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GROWTH OF EXCISED ROOTS OF THE TOMATO

WILLIAM J. ROBBINS AND MARY BARTLEY SCHMIDT

(WITH TWENTY FIGURES)

Introduction

This investigation[1] was undertaken to define the nutritional requirements of the root of a higher plant, *Lycopersicon esculentum* Mill. The underground root of a land plant contains no chlorophyll and grows in darkness. It depends upon the top for the carbohydrate needed in its development, and in this respect its relation to the top is that of a parasite to its host. Is the root dependent upon the green shoot for other essential materials?

ROBBINS (**10**) initiated a study of this question in 1917, and found it possible to grow excised root tips of corn (*Zea mays* L.) for considerable periods of time in solution cultures under sterile conditions. He and his coworkers (MANEVAL and V. B. WHITE), however, were unable to secure unlimited growth of the roots of this plant in any of the numerous media tested (**11, 12, 13, 14, 16, 17**).

P. R. WHITE (**29**) in 1934 reported unlimited growth of excised tomato roots in a medium of mineral salts, cane sugar, and yeast, a medium similar to that originally used by ROBBINS for corn roots. This discovery offered an opportunity to determine the nutritional requirements of the root of one kind of plant, since a medium which permits unlimited growth obviously contains all that the root re-

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quires for growth. A study of the growth of excised roots of tomato under sterile conditions was therefore begun in 1935.

Its purposes were: first, to confirm WHITE's report that unlimited growth of excised tomato roots is possible; second, to determine whether each of the three parts of his medium (the mineral salts, cane sugar, and yeast extract) was essential; third, to define as far as possible in what the essential character of these parts of his medium consisted. Particular attention was devoted to the yeast extract, which was the most complex and ill-defined part of the medium used by WHITE.

Each of the three portions was found essential. The mineral salts could not be omitted, nor could the cane sugar nor the yeast be omitted from the medium if growth was to be secured. The essential nature of the yeast was found to be determined by its content of vitamin B_1 or vitamin thiazole, although its ash content (perhaps its nitrogen content also) is of some importance. Other sources of carbon than cane sugar were discovered to be available, and certain inadequacies in the mineral nutrients in WHITE's solution were studied.

It was concluded that the tomato root probably depends upon the top not only for carbohydrates, but for vitamin B_1 (or thiazole). Notes on this investigation have been published from time to time (**15, 18, 19, 20**), and some of our results have already been confirmed by WHITE (**32, 33, 34**).

The significance from the standpoint of plant tissue culture of the development of a solution of known constituents in which unlimited growth of excised roots occurs is obvious. It permits a study of the mineral nutrition, nitrogen synthesis, energy relations, growth and development, and other fundamental physiological processes of a portion of a higher plant in a way hitherto impracticable. It is of some interest to note that while animal tissue culture was successfully used for many years before plant tissue culture had developed, a synthetic medium for animal tissue culture is still to be devised.

A review of the literature on the growth of excised roots in sterile culture or of plant tissue culture will not be attempted here. WHITE (**30**) has recently presented a review of this subject.

Material and methods

In these experiments excised roots of tomato were grown under sterile conditions in liquid media. The roots came originally from germinated seeds of a pink-fruited variety from Mexico, Ajo de Verrado no. 580, secured through Dr. J. W. LESLEY and Dr. H. L. BLOOD. This tomato is placed in the species *Lycopersicon esculentum* but it has some characteristics of *L. pimpinellifolium*. The fruits were surface sterilized, and seeds were removed from them and

TABLE 1

DATES OF INITIATION OF VARIOUS PASSAGES REFERRED TO IN TEXT

PASSAGE	BEGUN	PASSAGE	BEGUN
Original	Sept. 29, 1935	15	Nov. 24, 1936
1	Oct. 18	16	Dec. 26, 27
2	Nov. 5	17	Jan. 30, 31, 1937
3	Nov. 26	18	Mar. 5, 6
3a	Dec. 7	19	Apr. 6
4	Dec. 24	20	May 8
5	Jan. 30, 1936	21	June 9
6	Feb. 23	22	July 16
7	Mar. 20	23	Aug. 23
8	Apr. 11	24	Sept. 27
9	May 20	25	Oct. 19
10	June 20	26a	Nov. 15
11	July 18	26b	Nov. 26, 27
12	Aug. 20	27a	Dec. 14
13	Sept. 23	27b	Dec. 22
14	Oct. 21, 22	27c	Jan. 10, 1938

placed on sterile distilled water agar to germinate. After the roots were several centimeters long, 2 mm. tips were cut off and placed in flasks of the basic medium, WHITE'S solution (**29**).

All of the experimental work, unless otherwise noted, was performed with subcultures from two of these original excised roots, B and C. Subcultures were made at approximately monthly intervals resulting up to the present time in a total of twenty-seven passages from the original roots. Approximately 3000 subcultures have been made over a period of about $2\frac{1}{2}$ years. The dates for the passages are given in table 1.

The tomato root cultures represent two pure lines or clones. The results secured with these two clones did not differ significantly.

In making subcultures, pieces were cut from roots in the liquid

media by means of a fragment of a razor blade mounted in an aluminum handle. Each piece, except in a few special instances, had one or more growing tips. The cutting tool was sterilized by dipping it in 95% alcohol and burning off the alcohol. This prevented overheating of the blade or handle. Pieces of root were transferred to new culture media by means of a loop needle of chromium wire mounted in aluminum. The needles were sterilized in the flame and allowed to cool before touching the root fragment. All work involved in transferring roots was carried out in a steamed culture chamber, and contaminations were not frequent. Whenever a root became contaminated it was discarded.

The excised roots were grown individually in 40 or 50 ml. of solution in 125 ml. Erlenmeyer flasks of Pyrex glass.

The basic medium for these experiments was WHITE's solution (**29**), which has the following composition:

$Ca(NO_3)_2$	0.6	millimols	0.142 gm.
$MgSO_4 \cdot 7H_2O$	0.3		0.073
KNO_3	0.8		0.081
KCl	0.87		0.065
KH_2PO_4	0.09		0.012
$Fe_2(SO_4)_3$	0.006		0.0024
Redistilled water	1000.00		ml.

2% by weight of sucrose and 0.01% by weight (100 p.p.m.) of dried brewers' yeast. A filtered extract of the yeast was used.

WHITE's solution is an adaptation of the modified Pfeffer's solution originally used by ROBBINS (**10**) with $F_2(SO_4)_3$ replacing $FeCl_3$.

The sucrose used in preparing WHITE's solution was Pfanstiehl's c.p. sucrose, lot no. 409, except in the earlier experiments in which Merck's highest purity sucrose was used.

The dried brewers' yeast was strain K from Anheuser-Busch, St. Louis, Missouri. The yeast was extracted by boiling in redistilled water for $\frac{1}{2}$ hour, and centrifuging to bring down the solid material. The supernatant liquid was used.

Merck's synthetic vitamin B_1 (Betabion) was used most frequently. Preparations of the natural crystalline vitamin from Merck and the synthetic vitamin from the Winthrop Universal Co. were used also. No differences in the effects of these various preparations were noted.

Other solutions used frequently in these experiments were solution

W and solution Z. Solution W had the following composition: WHITE's mineral solution, 2% sucrose and 0.1 p.p.m. each of boron and zinc. Solution Z was composed of WHITE's mineral solution, 2% sucrose, and 1 ml. per liter of a modification of Hoagland's A to Z mixture. A modification of Hoagland's A to Z mixture was prepared by adding to 18 liters of water: LiCl 0.5 gm., $CuSO_4 \cdot 5H_2O$ 1.0 gm., $ZnSO_4$ 1.0 gm., H_3BO_3 11.0 gm., $Al_2(SO_4)_3 \cdot 18H_2O$ 1.0 gm., $SnCl_2 \cdot 2H_2O$ 0.5 gm., $MnSO_4 \cdot 4H_2O$ 7.0 gm., $NiCl_2 \cdot 6H_2O$ 1.0 gm., $Co(NO_3)_2$ 1.0 gm., $TiSO_4$ 1.8 gm., KI 0.5 gm., NaBr 0.5 gm.

The mineral salts were Merck's highest purity except for the calcium nitrate, which was Merck's chemically pure. Redistilled water was used without exception; the tap distilled water was distilled in Pyrex glassware at least once. All glassware used was Pyrex.

Nutrient media were sterilized in the autoclave at 120° C. for 20 minutes unless otherwise specified. For the first fourteen passages 40 ml. of liquid culture medium and for the remainder 50 ml. of medium per flask were used. Cultures were grown, unless otherwise stated, in diffuse light at room temperature or in water baths with the temperature controlled to some extent.

The effect of different treatments was judged by macroscopic observation at intervals during the growth of each set of cultures. This permitted approximations of rates of growth. Measurements of length were not made because of the profuse branching of the excised roots. Daily growth rates were not determined except on occasion, when sketches were made at intervals to estimate enlargement of fragments.

After about 2 months, those roots which had not been used as inoculum were removed from the culture flasks, rinsed in distilled water and dried. Air dry weights were taken as an indication of total growth. Since the best roots in each set were used for inoculating the next series, the dry weights secured were only an approximation of the total growth and were less than the maximum. All conclusions drawn from the data on dry weights were supported by the observations during the development of any particular set of cultures.

Experimental work

UNLIMITED GROWTH OF EXCISED TOMATO ROOTS.—Our first concern was to determine the validity of the report by WHITE (**29**) that

unlimited growth of excised tomato roots is possible. This report was confirmed in these experiments. Excised tomato roots were grown for about $2\frac{1}{2}$ years through twenty-seven passages in liquid media. The results for twenty passages in WHITE'S solution are summarized in table 2. The original tips were placed in WHITE'S

TABLE 2

RECORD OF TWENTY PASSAGES OF EXCISED TOMATO ROOTS IN WHITE'S SOLUTION

PASSAGE	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT PER ROOT (MG.)
Original	15	34.8	7.0–65.2
1	5	25.9	12.0–39.5
2	19	15.9	1.1–38.5
3	28	17.0	3.0–39.0
4	5	3.0	2.0– 3.5
5	15	16.2	2.0–43.0
6	13	11.0	2.0–17.5
7	19	18.5	8.3–33.0
8	8	13.3	4.0–34.0
9	8	28.9	12.6–42.0
10	6	26.0	23.0–32.3
11	7	14.8	7.0–19.5
12	6	22.3	17.0–29.2
13	6	8.5	4.5–11.9
14	15	7.3	4.2–12.1
15	5	7.8	3.3–10.4
16	28	5.4	0.9–10.4
17*	3	7.7	2.8–14.1
18	14	14.1	7.7–23.4
19	10	21.4	9.0–28.9
20	18	20.9	18.3–24.2

* Solution Z plus yeast extract used.

solution September 29, 1935. Transfers were made at approximately monthly intervals; the twentieth passage was made May 8, 1937.

While the data presented in table 2 confirm WHITE'S report of the possibility of unlimited growth of excised tomato roots, they also show that considerable variation in growth occurred during the period of the experiments. The original roots in WHITE'S solution grew better than those of any subsequent set, even though the root fragments used as inoculum were smaller for the original cultures than those used for later series (fig. 1). This suggests that the root fragments from the seedling root contained material which favored

their growth and which was not supplied in adequate quantity by the constituents of WHITE's solution. There was no indication of a progressive decrease in growth, however; in fact growth in passages 19 and 20 was as great as in passages 1 and 2.

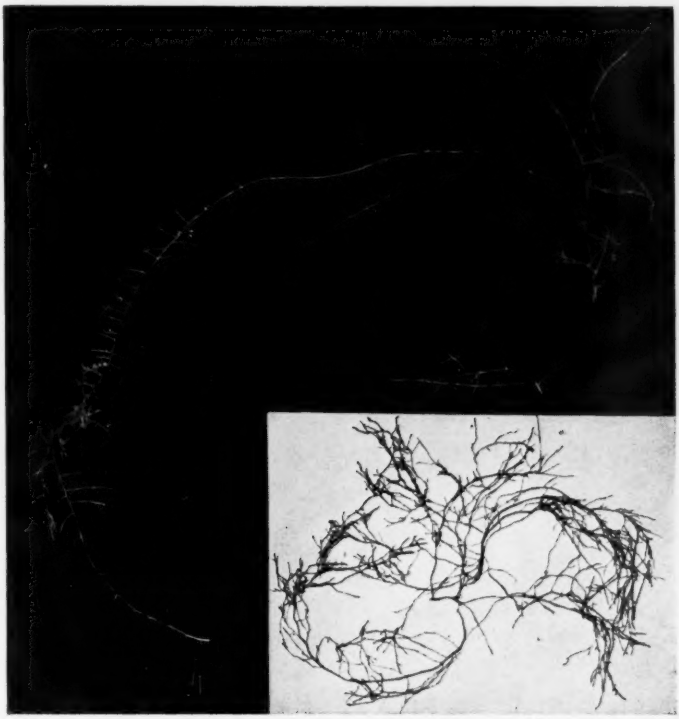


FIG. 1.—Original excised tomato root (above) and root from passage 19 (below). Both grown in WHITE's solution and shown on same scale.

A part of the considerable variation in growth in the successive passages was probably the result of differences in temperature of incubation. For example, the least growth occurred in passages 4 and 16, both of which were started in the month of December. WHITE (31) found a seasonal fluctuation in growth rate which he correlated with seasonal fluctuation in temperature.

In these experiments considerable variation among roots grown under the same conditions in a single passage was noted. This is shown by the range of the dry weights of individual roots for each passage as given in table 2. This variability in growth of roots in a given series grown under uniform conditions was noted throughout the experiments and is discussed later.

INDISPENSABILITY OF MINERAL SALTS, SUGAR, AND YEAST IN WHITE'S SOLUTION.—In addition to water and dissolved gases including oxygen, WHITE's solution is composed of three main parts: yeast extract, sugar, and a mixture of mineral salts. Is each of these

TABLE 3

DATA DEMONSTRATING INDISPENSABILITY OF YEAST, SUGAR AND MINERAL SALTS IN WHITE'S MEDIUM

PASSAGE	MODIFICATION OF WHITE'S MEDIUM	NO. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		USED	WEIGHED		
2.....	None	28	19	15.9	1.1–38.5
2.....	Yeast omitted	20	1	0.2	.....
3.....	None	14	8	10.3	4.0–20.0
3.....	Sugar omitted	9	7	0.04	.....
3.....	Minerals omitted	9	8	0.09	.....
18.....	None	20	14	14.1	7.7–23.4
18.....	Yeast omitted	15	15	0.57	0.2– 1.7

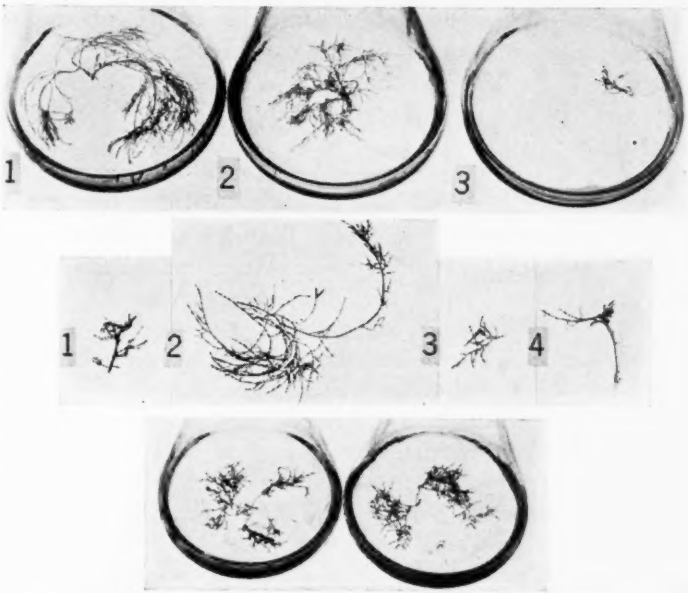
three parts essential for the growth of excised tomato roots? In our experiments little or no growth occurred in WHITE's medium from which any one of these three parts was omitted. The indispensable character of each of these constituents has been reported briefly (**18**) and confirmed by WHITE (**32**).

The indispensability of the yeast extract, the sugar, or the mineral salts is shown by the data in table 3. In passage 2, although but one root was weighed, none of the twenty grew appreciably in WHITE's solution with yeast omitted (fig. 2). Some roots grew slightly (maximum weight 1.7 mg.) in the solution lacking yeast in passage 18. This was believed to be at the expense of material carried over in the inoculum. In the absence of minerals or of sugar the growth was inappreciable (table 3).

Each part of the basic medium (WHITE's solution) was essential

for the growth of tomato roots in pure culture. The absence of any of the three portions resulted in complete failure to grow, not merely in a decrease in rate of growth.

YEAST AND GROWTH OF TOMATO ROOTS.—It was found in the experiments just described that the yeast in WHITE's solution was in-



FIGS. 2–4.—Fig. 2 (above), effect of absence of yeast extract on growth (passage 18): *1*, in WHITE's solution; *2*, in same solution with 10 gamma vitamin B_1 instead of yeast; *3*, in same solution without yeast or vitamin B_1. Fig. 3 (center), effect of yeast functions on growth: *1*, in WHITE's solution with absolute alcoholic extract of yeast; *2*, with 80% alcoholic extract; *3*, with subsequent water extract; *4*, with ash of residue. Second passage in media used in *1*, *2*, and *3*. Fig. 4 (below), unlimited growth in medium of mineral salts, cane sugar, and vitamin B_1. Two roots from twelfth passage in this medium.

dispensable for the growth of excised tomato roots. Why is yeast indispensable, and can any other natural or synthetic products be found which will replace the yeast portion of WHITE's solution? From our results it is believed that the indispensable character of the yeast

is because of its vitamin B_1 or thiazole[2] content. This statement does not imply that other constituents of the yeast are not beneficial. We have found, also, that yeast may be replaced in WHITE's solution by other natural products, including malt flour, some samples of maltose, and neo-peptone.

Yeast extract as used in our experiments was a mixture of substances, including mineral salts, nitrogenous compounds, various vitamins, and an unknown number of other organic compounds. There was a possibility that not all of the constituents of the yeast were essential to a satisfactory medium. In order to determine in what the essential nature of the yeast consisted, two procedures appeared possible: (a) fractionating the yeast to discover what portion of the yeast extract was essential; (b) substituting for yeast in WHITE's solution specific compounds known to be present in the yeast. Both methods were used in these investigations.

A. FRACTIONATION OF YEAST.—By successive extraction of yeast with absolute ethyl alcohol, 80% alcohol, and water it was found that the beneficial factor, or factors, were but slightly soluble in absolute alcohol and almost entirely removed by 80% alcohol.

The dried brewers' yeast was fractionated as follows: 1 gm. of dried yeast was mixed with about 100 ml. of absolute alcohol, allowed to stand for 10–15 minutes, mixed thoroughly and filtered through Whatman no. 50 (hardened) filter paper. The filtrate was evaporated to dryness and boiled with 100 ml. of redistilled water. The residue from the absolute alcohol extraction was washed on the filter paper several times with 80% alcohol, and the filtrate, yellow in color, was treated as the absolute alcoholic extract was treated. The residue left by these two extractions was washed several times with hot redistilled water and the filtrate evaporated to 100 ml. The final residue was removed from the filter paper and ashed at low-red heat in a muffle furnace. The ash was suspended in 100 ml. of redistilled water. Each of the four fractions was added to WHITE's solution lacking yeast in amounts equivalent to 100 p.p.m. of yeast.

[2] When the terms thiazole or pyrimidine are used in this paper, 4-methyl-5-hydroxyethylthiazole or the 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide is meant. These compounds were used by WILLIAMS and CLINE (37) in synthesizing vitamin B_1.

The effect of the fractions is illustrated by the data in table 4. In passage 11, begun July 18, 1936, thirteen subcultures were made in WHITE's solution and six in each of the following: WHITE's medium with yeast replaced by the absolute alcoholic extract; by the 80% alcoholic extract; by the subsequent water extract; by the ash of the residue. These roots showed that the medium containing the 80% alcoholic extract was much superior to those containing the other

TABLE 4

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION, AND SAME SOLUTION WITH YEAST REPLACED BY VARIOUS YEAST FRACTIONS. SUBCULTURES OF ROOTS GROWN IN PASSAGE 11 USED FOR PASSAGE 12, EXCEPT FOR THOSE IN MEDIUM CONTAINING ASH

PASSAGE	YEAST FRACTION IN WHITE'S SOLUTION	NO. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		GROWN	WEIGHED		
11	Usual water extract	13	7	14.8	7.0–19.5
	Absolute alcohol extract	6	3	3.1	2.3– 4.0
	80% alcohol extract	6	5	8.5	1.1–11.0
	Subsequent water extract	6	4	5.8	4.0– 9.0
	Ash of residue	6	6	1.4	0.2– 5.0
12	Usual water extract	6	6	22.5	17.0–29.2
	Absolute alcohol extract	6	5	1.1	0.4– 2.4
	80% alcohol extract	6	6	8.6	5.3–10.2
	Subsequent water extract	6	6	1.1	0.7– 1.9
	Ash of residue	6	5	1.7	0.7– 2.2

fractions. Growth in the medium containing the absolute alcoholic extract, or the water extract, was slight; in that containing the ash of the residue almost no growth occurred.

Subcultures were made (passage 12) from the roots grown in the media containing each fraction of the yeast except that containing the ash, in which not enough growth occurred to furnish inoculum. Growth of subcultures in the medium containing the 80% alcoholic extract was comparable with that in passage 11, but almost no growth occurred in media containing the absolute alcoholic extract and the water extract. Typical roots are shown in figure 3.

Growth of the roots in the media containing the various yeast fractions showed that the active principle in yeast could be largely

removed by treatment with 80% alcohol. The 80% alcoholic extract was not so effective as the water extract of yeast customarily used in preparing WHITE's solution. However, enough of the essential portion was removed by alcoholic extraction so that a subsequent water extract was not adequate for continued growth.

B. VITAMIN B_1 AND GROWTH OF TOMATO ROOTS.—Vitamin B_1 is a constituent of dried yeast, is soluble in 80% alcohol and in water, and sparingly soluble in absolute alcohol (**18**). SCHOPFER (**23**) demonstrated the essential nature of vitamin B_1 for the growth of a mold, *Phycomyces blakesleeanus* Burgeff. Experiments were therefore initiated October 22, 1936, to determine the effect of this vitamin on the growth of excised tomato roots. These experiments showed that unlimited growth of tomato roots will probably occur in a solution of mineral salts, chemically pure sucrose, and vitamin B_1. Excised tomato roots have been carried through fourteen passages over a period of more than 14 months in this medium. The effect of the vitamin as used here was not so great as that of the yeast extract; but as a substitute for yeast in WHITE's solution it was adequate for continued growth at a low level. The effect of replacing the yeast in WHITE's solution by vitamin B_1 is shown in the following experiments.

Twenty-seven subcultures were made in WHITE's solution in passage 14, and eleven in the same medium with vitamin B_1, 10 gamma per flask, replacing the yeast. From these roots subcultures were continued through fourteen passages in a medium of mineral salts, Pfanstiehl's c.p. sucrose no. 409, and vitamin B_1. The dry weights of roots for twelve of these passages are given in table 5. For each passage the best roots were used as material for inoculating the solutions used in the succeeding passage. The dry weights given in table 5 do not therefore equal the maximum average dry weight per root for each passage. From these data, as well as observations made during the period covered by the experiments, it appears that unlimited growth of excised tomato roots is possible in a medium of mineral salts, cane sugar, and vitamin B_1. Figure 4 shows two roots from the twelfth successive passage in this medium. There was no constant decrease in growth as would be anticipated were this medium inadequate. Variations in growth occurred, due in part to differences

in incubation temperatures. For example, the temperatures in passages 22 to 24 were lower than those for the other passages. The variations were also the result in part of differences in the mineral salts used. For example, in passages 20 to 25, the salts of WHITE's solution were supplemented in the solutions containing vitamin B_1 by the addition of 0.1 p.p.m. of B as boric acid and 0.1 p.p.m. of Zn as zinc sulphate. These supplements and a higher temperature im-

TABLE 5

GROWTH OF ROOTS IN SUCCESSIVE PASSAGES IN WHITE'S SOLUTION, AND IN SAME SOLUTION WITH YEAST REPLACED BY VITAMIN B_1
10 GAMMA PER FLASK

PASSAGE	WHITE'S SOLUTION		WHITE'S SOLUTION WITH VITAMIN B_1 REPLACING YEAST		
	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
14	9	6.7	8	4.5	0.8– 7.3
15	5	7.8	7	7.1	2.7–17.1
16	28	5.4	5	4.6	1.6– 5.5
17	3	7.7	7	4.0	1.4– 5.6
18	14	14.1	7	4.4	0.9–10.4
19	10	21.4	9	7.5	2.6–23.1
20*	4	22.0	14	11.5	1.9–26.7
21*	(not used)		6	16.5	10.7–25.4
22*†	(not used)		6	7.5	5.2– 8.2
23*†	(not used)		7	5.6	0.4– 9.6
24*†	(not used)		5	3.0	2.1– 5.4
25*	3	8.7	4	8.3	6.0–11.2

* 0.1 p.p.m. boron and 0.1 p.p.m. zinc added to medium containing vitamin B_1.
† Roots grown at about 16° C. (others grown at room temperature).

proved the growth, as shown by the dry weights in passages 20 and 21. Nevertheless, the growth in WHITE's solution was superior to that in the same solution with vitamin B_1 replacing the yeast. This is discussed in more detail later.

C. AUTOCLAVED YEAST, AUTOCLAVED VITAMIN B_1, AND GROWTH OF TOMATO ROOTS.—The solutions in which yeast extract or vitamin B_1 was used in these experiments were heated for 20 minutes at 120°C. It was assumed that this period of heating in an acid solution (pH 5.0 to 5.2) did not destroy the vitamin. Furthermore we used vita-

min B_1 which had been filtered sterile and secured results similar to those obtained with the vitamin solutions which had been sterilized by heating. However, vitamin B_1 in alkaline solution is largely destroyed by long continued high temperatures (**36**). If vitamin B_1 were the constituent of yeast extract essential for the growth of tomato roots, then the growth promoting effect should be destroyed by sufficient heating in alkaline suspension; and vitamin B_1 autoclaved in alkaline solution should lose its growth promoting

TABLE 6

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION AND IN SAME SOLUTION WITH USUAL YEAST EXTRACT REPLACED BY YEAST EXTRACT FROM: YEAST HEATED AT 120° C. FOR 5 HOURS OR FOR 12 HOURS AT INITIAL PH 8.9, BY VITAMIN B_1, OR BY VITAMIN B_1 HEATED AT 120° C. FOR 12 HOURS AT INITIAL PH 8.9

PASSAGE	SPECIAL TREATMENT OF WHITE'S SOLUTION	NO. ROOTS		DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
		USED	WEIGHED		
15.....	None	30	5	7.8	3.3–10.4
	Yeast, 120° C. for 5 hours	10	7	10.3	3.8–15.4
16.....	None	40	28	5.4	0.9–10.4
	Yeast, 120° C. for 12 hours	10	9	14.3	9.3–19.9
	Vitamin B_1	10	5	4.6	1.6– 5.5
	Vitamin B_1, 120° C. for 12 hours	10	9	4.7	0.7– 9.5

properties. In our experiments, however, the effectiveness of yeast autoclaved at 120° C. for 5 hours at initial pH 8.0 and at 120° C. for 12 hours at the same initial pH was increased. Furthermore vitamin B_1 autoclaved 12 hours at 120° C. at initial pH 8.0 was still active.

The effect of heating yeast or vitamin B_1 is illustrated in table 6. The yeast suspension from which the extract was made in passage 15 was heated for 5 hours at 120° C. and in passage 16 for 12 hours at 120° C. The vitamin was heated for 12 hours at 120° C. in passage 16. In each instance the original reaction of the yeast suspension or the vitamin solution was adjusted to pH 8.9 before heating. At the end of the period of heating, however, the reaction had returned to approximately its original value of pH 5.1.

In both passages the extract made from the yeast which had been autoclaved for several hours was superior in its effect to that which had been prepared by boiling. Furthermore the effect of the yeast which had been heated for the longer period was somewhat greater (table 6). The greater effect of the yeast extract which had been heated for several hours as compared with that prepared as usual by boiling for a few minutes might be the result of destroying some injurious materials in the extract or of producing some beneficial decomposition products. We are inclined to believe, however, that it is probably associated with the more complete extraction from the yeast of substances which may be inorganic in character. This hypothesis is favored because of our conclusion, presented later, that the mineral salts in WHITE's solution are not adequate for the best growth of tomato roots, at least as we have used it.

In any event these experiments suggested that other substances might replace vitamin B_1 in the growth of tomato roots.

D. INTERMEDIATES OF VITAMIN B_1 AND GROWTH OF TOMATO ROOTS.—The failure of prolonged autoclaving in alkaline solution to destroy the growth promoting effects of yeast or of vitamin B_1 raised the question as to whether other substances replace vitamin B_1 in the growth of excised tomato roots.

WILLIAMS and CLINE (**37**) synthesized vitamin B_1 from a pyrimidine and a thiazole. Through the courtesy of Dr. R. R. WILLIAMS and of Merck and Co., samples of 4-methyl-5-hydroxy-ethylthiazole, of 2-methyl-5-bromomethyl-6-aminopyrimidine hydrobromide, and of 2-methyl-5-ethoxymethyl-6-aminopyrimidine were secured. It was found that for the growth of tomato roots vitamin B_1 could be replaced by the two intermediates or by the thiazole alone. This was demonstrated by the following experiments.

In passage 19 (begun April 11, 1937) six transfers were made to solution W and five transfers to solution W, with each of the following supplements: 5 gamma of thiazole per flask, 5 gamma of ethoxypyrimidine per flask, 5 gamma of bromopyrimidine per flask, 5 gamma each of ethoxypyrimidine and thiazole, 5 gamma each of bromopyrimidine and thiazole, and 5 gamma of vitamin B_1. Growth occurred in all cultures which contained either vitamin B_1 or thiazole. No growth occurred in solution W, nor in the same solutions

supplemented with either the ethoxypyrimidine or the bromopyrimidine. Roots grew in the solutions supplemented with vitamin B_1, thiazole, and with the mixtures of thiazole and either pyrimidine (table 7). Growth in the mixture of thiazole and pyrimidine showed that the failure of roots to grow in the solutions supplemented with the pyrimidines alone was not because of the toxicity of the latter solutions. All roots were used in the dry weight determinations except the best root in the thiazole series, which was used as

TABLE 7

DRY WEIGHTS OF ROOTS FROM PASSAGE 19 GROWN IN SOLUTION W AND IN SAME SOLUTION WITH VITAMIN B_1 OR INTERMEDIATES OF VITAMIN B_1

ADDITIONS TO SOLUTION W	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
None	6	0.6	0.3– 0.7
Vitamin B_1	5	6.95	2.0–17.3
Thiazole	4	3.6	1.2– 8.2
Thiazole and ethoxypyrimidine	5	4.0	2.0– 8.1
Thiazole and bromopyrimidine	5	4.3	2.1–13.5
Ethoxypyrimidine	5	0.8	0.4– 1.6
Bromopyrimidine	5	0.7	0.2– 1.5

an inoculum for the subsequent transfer to solutions containing thiazole.

Since the original experiment with thiazole, the tomato roots have been maintained in solution W plus thiazole through eight successive passages extending over 8 months. The roots in the solution containing mineral salts, cane sugar, and thiazole give every indication that unlimited growth of excised tomato roots is possible in this medium. In fact (table 8) the growth in the solutions supplemented with thiazole alone appeared to be somewhat superior to that in the solution supplemented with vitamin B_1 (fig. 5).

We are not prepared to explain the apparent superiority of the thiazole at 10 gamma per flask over the vitamin B_1. It may have been an effect of some beneficial impurity in the thiazole or the fact that the vitamin B_1 and the thiazole were not used in molecularly equivalent quantities. In any event the important finding was the

apparent possibility of unlimited growth in a solution consisting of mineral salts, cane sugar, and the vitamin thiazole.

The growth of excised tomato roots in solutions with thiazole replacing the yeast was characteristic. Various other solutions gave other characteristic types of growth; these will be discussed in more detail later. Figure 5 shows a typical root in solution W plus thiazole; the branching was profuse, with the main branches long and the secondary branches somewhat short. This root differs markedly

TABLE 8

DRY WEIGHTS OF ROOTS THROUGH SEVEN PASSAGES IN SOLUTION W WITH 10 GAMMA OF VITAMIN B_1 PER FLASK, AND IN SAME SOLUTION WITH 10 GAMMA OF THIAZOLE PER FLASK

Passage	Solution W plus vitamin B_1		Solution W plus thiazole		
	No. roots weighed	Dry weight per root (mg.)	No. roots weighed	Dry weight per root (mg.)	Range dry weight (mg.)
19*	5	7.0	4	3.6	1.2– 8.2
20	14	11.5	4	13.7	.....
21	6	16.5	2	24.6	23.8–25.5
22†	6	7.5	2	12.9	11.7–14.1
23†	7	5.6	4	17.3	13.7–21.8
24†	5	3.0	4	23.1	14.3–32.9
25	4	8.3	6	11.9	8.1–14.5

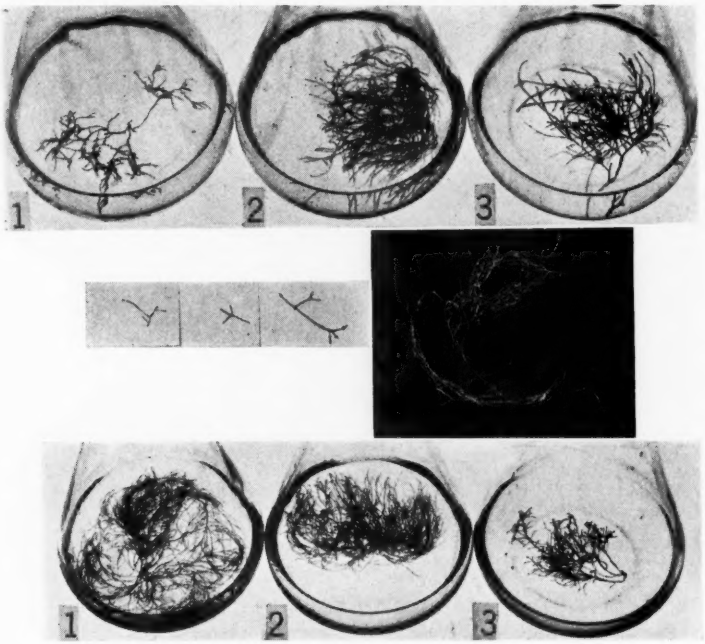
* Five gamma each of supplements used.

† Roots kept at approximately 16° C. (others grown at room temperature).

from the other two roots shown in figure 5. The root in solution W plus B_1 is also typical, and different in appearance from the thiazole root. It should be noted that one of these two solutions contained 0.2 gamma of vitamin B_1 per ml. and the other 0.2 gamma of thiazole.

Does the tomato root require thiazole alone or does it require vitamin B_1, synthesizing under the conditions of our experiments sufficient pyrimidine for growth but an inadequate amount of thiazole? We are of the opinion that the tomato root requires the vitamin and synthesizes the necessary pyrimidine but forms little or no thiazole. This assumption is supported by the fact that tomato roots grown in a solution supplemented with thiazole alone form pyrimidine.

E. SYNTHESIS OF PYRIMIDINE BY TOMATO ROOTS.—The synthesis by excised tomato roots of a pyrimidine which will form a portion of the vitamin B_1 molecule was demonstrated by the use of *Phycomyces blakesleeanus*. This fungus requires for growth an external supply of



FIGS. 5–7.—Fig. 5 (above), thiazole and growth of tomato roots (passage 23): *1*, solution W plus vitamin B_1; *2*, WHITE'S solution with Eastman's maltose instead of cane sugar; *3*, solution W plus thiazole. Fig. 6 (center), effect of indole(3)acetic acid in dark. Left to right: in WHITE'S solution plus 1 p.p.m. acid; plus 0.1 p.p.m.; plus 0.01 p.p.m.; plus 0.001 p.p.m. Fig. 7 (below), effect of malt flour; WHITE'S medium with 100 p.p.m. extract of malt flour instead of yeast: *1*, fresh extract added four times; *2*, solution replaced four times; *3*, undisturbed.

vitamin B_1 or of both intermediates. One intermediate (either the pyrimidine or thiazole alone) is inadequate (**21**). Tomato roots were grown in solution W supplemented with 10 gamma of vitamin thiazole per flask. At the end of approximately 1 month of growth the roots were removed from the solutions, dried, and weighed. By

suitable additions the solutions in which the roots had grown were adjusted to contain 0.5% $MgSO_4 \cdot 7H_2O$, 1.5% KH_2PO_4, 1% asparagine, and 10% dextrose. The solutions were sterilized and inoculated with *Phycomyces*. A small amount of submerged mycelium developed, the dry weight of which indicated the presence in the solution in which a tomato root had grown of between 0.003 and 0.015 gamma of pyrimidine. The dried roots were powdered and added to a solution of the composition just given. The solutions were sterilized and inoculated with *Phycomyces*. The resulting growth indicated the presence in each root of between 0.3 and 1.0 gamma of vitamin pyrimidine. It appears therefore that tomato roots grown in a solution of mineral salts, sugar, and vitamin thiazole synthesize a pyrimidine which will form a portion of vitamin B_1.

F. SUBSTITUTES FOR YEAST OTHER THAN VITAMIN B_1 AND ITS INTERMEDIATES.—The experiments show that the indispensable character of the yeast is probably related to its content of vitamin B_1 or intermediates, especially thiazole. We have been interested in determining whether any other specific compounds or natural products would replace the yeast. No specific compound other than vitamin B_1 or thiazole was found. However, malt flour, maltose, and peptone were used successfully as substitutes for yeast.

(a) INDOLE(3)ACETIC ACID.—Concentrations of 1, 5, and 15 p.p.m. were used as a substitute for the yeast extract in WHITE'S solution. Growth in the solutions containing indole(3)acetic acid was inappreciable. While this experiment did not suggest that the acid would replace yeast, it is possible that it was not used at sufficient dilution since stimulation of root growth has been reported at greater dilutions than were used here (**4, 7, 26**).

Indole(3)acetic acid was used also as a supplement to WHITE'S solution. It completely inhibited the growth of excised tomato roots in the dark at concentrations of 1, 0.1, and 0.01 p.p.m. No effect was apparent at a concentration of 0.001 p.p.m. (fig. 6). In the light almost no growth occurred in WHITE'S solution containing 15 or 5 p.p.m.; about 50% reduction in growth was produced by 1, 0.1, or 0.01 p.p.m. Growth in the light at concentrations of indole(3)-acetic acid which completely inhibited growth in the dark was because light destroyed the acid. Root fragments grew normally in

WHITE'S solution containing 1 p.p.m. of indole(3)acetic acid which had stood in the light for 1 month under sterile conditions. This concentration completely inhibited growth in the dark, and when used a day or two after preparation reduced growth in the light 50% or more.

(b) YEAST ASH.—The ash of an amount of yeast equivalent to that customarily used in WHITE'S medium was not found to substitute for the yeast.

(c) FILTER PAPER.—ROBBINS and WHITE (**16**) found filter paper to be beneficial to the growth of excised corn roots, but it did not substitute for yeast in WHITE'S solution. In our experiments $\frac{1}{2}$ sheet of A. H. Thomas qualitative filter paper was used per flask in place of the yeast extract.

(d) VITAMIN C OR ASCORBIC ACID.—Vitamin C was used at a concentration of 10 gamma per flask in place of the yeast extract in WHITE'S solution. It did not substitute for yeast in this medium.

(e) PANTOTHENIC ACID.—Pantothenic acid was used without success as a substitute for the yeast in WHITE'S solution. Two samples of the acid were secured from R. J. WILLIAMS, who had found this substance beneficial to certain strains of yeast (**35**). In passage 16, these two samples were used at 10 gamma per flask in WHITE'S medium without yeast, in two sets of ten subcultures each. The roots grew to some extent, but no appreciable growth was obtained when subcultures were made from the best roots in these two sets into more of the same media in passage 17.

(f) LACTOFLAVIN, VITAMIN B_2, OR VITAMIN G.—Lactoflavin was used without success as a substitute for yeast. In passage 17, ten subcultures were made in solution Z plus 10 gamma of lactoflavin per flask and ten in the same solution supplemented with vitamin B_1.[3] The roots in the solution with lactoflavin alone did not grow; those with lactoflavin and B_1 grew as well as roots in solution Z plus vitamin B_1. The failure of lactoflavin to substitute for yeast was not related to an injury caused by the lactoflavin.

(g) ASPARAGINE.—Asparagine at 100 p.p.m. did not substitute for the yeast extract. In passage 17, ten subcultures were made in

[3] Whenever a specific concentration of vitamin B_1 is not stated, the concentration used was 10 gamma per flask or 0.2 gamma per ml.

solution Z plus 100 p.p.m. asparagine, and ten in the same solution supplemented with vitamin B_1. Abnormal browning of the roots in the medium containing both asparagine and vitamin B_1 indicated that this amount was slightly injurious.

(h) CYSTEINE HYDROCHLORIDE.—Cysteine hydrochloride, considered a growth stimulant by SCHEITTERER (**22**) and by GAUTHERET (**6**), did not substitute for the yeast. In passage 17, ten root fragments were cultivated in solution Z plus 10 gamma of cysteine hydrochloride per flask, and ten in the same solution plus vitamin B_1. Roots in the solution with cysteine hydrochloride alone did not grow; those in the solution containing vitamin B_1 in addition grew appreciably.

(i) INOSITOL.—Inositol (inosite) has been found to be favorable for the growth of yeast (**35**). Used at a concentration of 100 p.p.m., inositol proved a partial substitute for yeast although the growth obtained was not so good as that secured with 10 gamma of vitamin B_1 per flask. The possibility that the inositol contained vitamin B_1 or thiazole as a contaminant was not investigated, and no subcultures were made.

(j) UREA.—Urea used at 100 p.p.m. injured the excised tomato roots. Root fragments transferred to solution Z containing urea and vitamin B_1 did not grow. In this same solution without urea appreciable growth occurred.

(k) PIMELIC ACID.—Pimelic acid, found to be a growth substance for bacteria by MUELLER (**8**), was used unsuccessfully as a substitute for yeast. Pimelic acid at 10 gamma per flask and pimelic acid plus vitamin B_1 were used as a substitute for the yeast extract. No growth occurred with the pimelic acid but normal growth was secured with the mixture of pimelic acid and vitamin.

(l) NUCLEIC ACID.—An acid hydrolysate of nucleic acid at 10 gamma per flask proved unsatisfactory as a substitute for the yeast extract. The growth in a medium containing thiazole or vitamin B_1 and the nucleic acid hydrolysate instead of yeast extract was not quite so good as in a medium containing vitamin B_1. While the nucleic acid hydrolysate was somewhat injurious, failure of growth in the medium in which it replaced the yeast was evidently not the result of the toxicity of the nucleic acid hydrolysate.

(m) MERCK MALTOSE NO. 22068.—In the study of the availability of various sources of carbon, which is discussed later, several samples of maltose were used. Some of them proved to substitute for yeast in addition to acting as a source of carbon. Growth in the solutions in which maltose was substituted for cane sugar and yeast was not so great as in WHITE'S solution. Not all samples of maltose, even from the same company, were equally effective; some had no effect and others proved actually injurious. It is assumed that the active maltose contained either vitamin B_1 or a substitute for the vitamin, a finding which is not surprising in view of the results secured by SCHOPFER (**23**) on the growth of *Phycomyces* in maltose solutions.

Contrary to our expectations, however, the beneficial material in maltose was largely removed by treatment with Fuller's earth but not by charcoal. This may indicate that the beneficial material was neither vitamin B_1 nor thiazole, since we would anticipate that these substances would be adsorbed by both adsorbents (**25**); it may have been the result of the procedure followed in using the adsorbents.

An active maltose was treated with Fuller's earth or Merck's medicinal charcoal (4 gm. of the adsorbent to 10 gm. of sugar in solution). The untreated maltose or the treated maltose was used to replace the yeast and cane sugar in WHITE'S solution. In addition a water extract of the charcoal was added to the medium containing the untreated maltose; the untreated maltose at 100 p.p.m. was also used to replace the yeast in WHITE'S medium. Some growth occurred in the solution containing 2% maltose instead of cane sugar and yeast, indicating the presence of vitamin B_1, thiazole, or a substitute therefor. The amount of the growth substance in the maltose was considerably less than in the yeast extract, since no growth occurred in the solutions with yeast replaced by 100 p.p.m. of maltose. The treatment with Fuller's earth removed the beneficial material but the addition of vitamin B_1 to the treated maltose permitted growth to occur. This suggests that the effect of the Fuller's earth was to remove vitamin B_1 from the maltose. Treatment of the maltose with charcoal improved growth somewhat, however, although still further improvement was noted when vitamin B_1 was added to the maltose treated with charcoal. Either the active material in the maltose was not vitamin B_1 (or thiazole) or the treatment with charcoal for some reason did not remove it as anticipated.

(n) MALT FLOUR.—The favorable effects observed with some samples of maltose suggested the use of malt flour as a substitute for yeast. At suitable concentrations it was found to be superior to the yeast extract. The malt flour was prepared by crushing malt in a press under about 12,000 pounds pressure and collecting the flour which could be rubbed through a fine sieve. Malt flour extract was prepared as was the yeast extract; the flour was boiled for approximately one-half hour in redistilled water; the suspension was centrifuged and sufficient of the supernatant liquid was used to be equivalent to 100 p.p.m. of the malt flour in the culture solution.

TABLE 9

DRY WEIGHTS OF ROOTS FROM PASSAGE 18, GROWN IN WHITE'S SOLUTION WITH WATER EXTRACT OF 100 P.P.M. MALT FLOUR REPLACING YEAST EXTRACT

TREATMENT	AGE OF ROOTS (MONTHS)	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)
Undisturbed	2	1	11.4
Solution replaced four times	2	1	30.2
Malt flour extract added four times	2	1	35.0
Undisturbed	4	2	15.2
Solution replaced four times	4	2	52.2
Malt flour extract added four times	4	2	71.0

The excised roots grew rapidly for about 10 days in the medium containing an extract of malt flour and then ceased growth. This suggested that something in the flour was exhausted by the roots, resulting in a cessation of growth. This assumption was substantiated by noting the beneficial effects of adding fresh quantities of malt flour to a solution in which growth had ceased and by replacing the solution with a fresh solution containing extract of the flour. These results are illustrated by the data in table 9 and by figure 7.

Fifteen subcultures were made in WHITE'S medium with 100 p.p.m. of extract of malt flour in place of the yeast. Five of these subcultures were left undisturbed; for five the solution was poured off and fresh solution added four times at intervals of about 2 weeks; and to five, fresh malt flour extract was added four times at 2 week intervals. At the end of 2 months the dry weight of a root from those solutions receiving fresh supplies of malt flour was about

three times that of a root which had had but one lot of malt flour. The difference at the end of 4 months was still greater (table 9).

The material in the malt flour which limits the growth of the tomato roots appeared to be vitamin B_1 or a substitute therefor. This followed because the addition of vitamin B_1 to malt flour markedly improved growth, and because treatment of malt flour

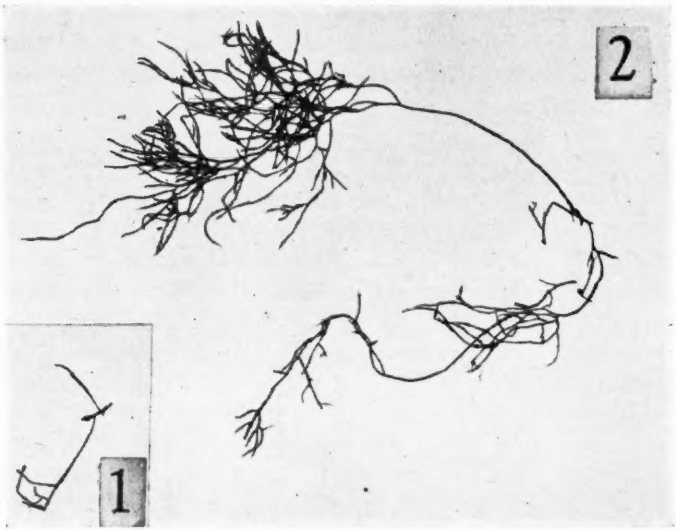


FIG. 8.—Effect of treatment of malt flour with charcoal: *1*, in WHITE'S solution with yeast replaced by extract of malt flour treated with charcoal; *2*, in same solution plus vitamin B_1.

with charcoal removed the active material. The addition of vitamin B_1 to cultures containing malt flour treated with charcoal permitted some growth, although apparently other beneficial materials also were removed by the charcoal (fig. 8).

(o) PEPTONE.—ROBBINS (11) found peptone favorable for the growth of excised corn roots. We found that at a sufficiently high concentration peptone was a substitute for yeast for the growth of tomato roots. Neo-peptone (Digestive Ferments Co.) was used at two concentrations, 10 and 100 p.p.m., as a substitute for yeast.

Growth of excised tomato roots occurred in the solutions in which 100 p.p.m. peptone replaced the yeast, but very little growth occurred with the 10 p.p.m. peptone (table 10 and figure 9). Since the addition of vitamin B_1 to the solution containing 10 p.p.m. peptone, but no yeast, markedly improved growth, we believe that the vitamin B_1 (or thiazole) content of the peptone was the limiting factor in determining growth in the presence of peptone. It is estimated that the 100 p.p.m. of peptone supplied 0.001 gamma or less of vitamin

TABLE 10

DRY WEIGHTS OF ROOTS FROM PASSAGE 24 IN SOLUTION W WITH PEPTONE, PEPTONE AND VITAMIN B_1, OR THIAZOLE. ALL ROOTS TRANSFERRED FROM EASTMAN'S MALTOSE CULTURES EXCEPT THOSE IN THIAZOLE, WHICH WERE IN SEVENTH PASSAGE IN THIS SOLUTION

ADDITIONS TO SOLUTION W	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
Peptone 10 p.p.m.	5	1.3	0.6– 1.6
Peptone 10 p.p.m. and vitamin B_1	5	10.8	8.2–12.8
Peptone 100 p.p.m.	5	7.6	5.0– 8.8
Peptone 100 p.p.m. and vitamin B_1	5	18.3	15.2–20.6
Thiazole	4	23.1	14.3–32.9

B_1; that is, the vitamin B_1 content of peptone, judging from our results, was 0.00002% or less.

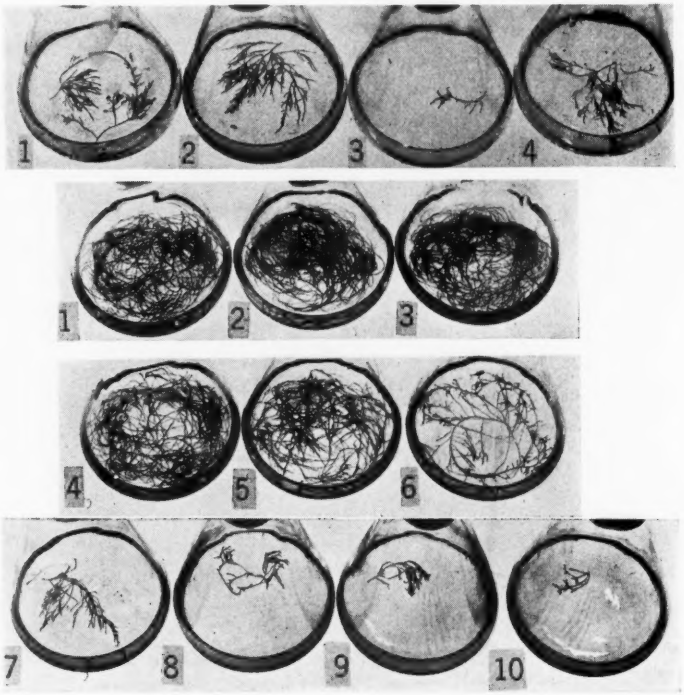
CONCENTRATION OF VITAMIN B_1 AND INTERMEDIATES

Since vitamin B_1 or thiazole was found to substitute for yeast in permitting unlimited growth of excised tomato roots, it was considered advisable to determine the effect of dilution of the vitamin and of its intermediates. Various dilutions were used in WHITE'S solution lacking yeast, in solution W, and in WHITE'S solution with cane sugar and yeast replaced by a light brown commercial sugar, or by the same sugar treated with charcoal.

In passage 20, WHITE'S solution was used with the cane sugar and yeast replaced by light brown sugar and vitamin B_1. Quantities of the vitamin from 100 gamma to 0.000001 gamma per flask containing 50 ml. of solution were used. The results of the experiment,

which ran for 9 weeks, are given in table 11 and photographs of typical roots are shown in figure 10.

A definite, although small, effect of 0.000001 gamma of vitamin B_1 was observed. The roots in flasks containing this quantity of the



FIGS. 9, 10.—Fig. 9 (above), effect of peptone (passage 24): *1*, in solution W plus 100 p.p.m. peptone; *2*, in same solution plus 10 gamma vitamin B_1; *3*, in solution W plus 10 p.p.m. peptone; *4*, in same solution plus 10 gamma vitamin B_1. Fig. 10 (below), effect of different amounts of vitamin B_1 (passage 20). Growth in WHITE'S solution with yeast and cane sugar replaced by light brown commercial sugar plus: *1*, 100 gamma vitamin B_1; *2*, 10 gamma; *3*, 1 gamma; *4*, 0.1; *5*, 0.01; *6*, 0.001; *7*, 0.0001; *8*, 0.00001; *9*, 0.000001; *10*, none.

vitamin averaged 3.6 mg., while those in the solutions containing no vitamin averaged 1.5 mg. The dilution of the vitamin for these solutions was 1 in 40,000,000,000,000. Dividing the increase in dry

weight (2.1 mg.) by the amount of vitamin involved, it is found that in the flasks containing 0.000001 gamma of vitamin 1 part of the vitamin was concerned in producing over 2,000,000,000 parts of dry matter. So far as we are aware, a physiological effectiveness as great as indicated by these figures has not previously been reported.

The maximum effectiveness of the vitamin in this medium occurred at about 0.1 gamma per flask. Further increases of the vitamin up to 100 gamma gave no consistent increase in dry weight.

TABLE 11

AVERAGE DRY WEIGHTS OF ROOTS FROM PASSAGE 20, GROWN IN WHITE'S MINERAL SOLUTION AND 2% LIGHT BROWN SUGAR WITH VARIOUS CONCENTRATIONS OF VITAMIN B_1 OR OF THIAZOLE

Amount supplied in gamma per flask	Vitamin B_1		Thiazole	
	No. roots weighed	Dry weight per root (mg.)	No. roots weighed	Dry weight per root (mg.)
100.0	2	73.4	4	30.4
10.0	3	55.8	4	18.0
1.0	2	82.4	4	9.2
0.1	4	72.3	4	4.8
0.01	4	57.3	4	3.4
0.001	4	18.1	.....	.....
0.0001	4	5.0	.....	.....
0.00001	4	4.2	.....	.....
0.000001	4	3.6	.....	.....
None	4	1.5	4	1.5

This is clear from the curve in figure 11. Evidently some factor other than the amount of vitamin B_1 limited the growth at the higher concentrations. It was noted that the growth of the individual roots at a given concentration of vitamin B_1 was quite uniform up to that of those in solutions containing 1 gamma of the vitamin. At 1, 10, and 100 gamma, considerable variation occurred and the best roots only were used in the dry weights given in table 11. The variation, which we are not prepared to explain, may be illustrated as follows: In the solution containing 1 gamma per flask two roots averaged 82.4 mg. and two others 27.2 mg.; at 10 gamma per flask three roots averaged 55.8 mg. and one weighed 19.5 mg.; at 100 gamma two roots averaged 73.4 mg. and two others 39.9 mg.

Amounts of thiazole ranging from 0.01 to 100 gamma per flask were used in the medium with light brown sugar. The growth in the thiazole cultures was not so good as in the corresponding cultures containing vitamin B_1. This is evident from the data in table 11, the photographs in figure 12, and the curves in figure 11. However, a definite effect from 0.01 gamma of thiazole was observed (3.4 mg. of dry matter as compared with 1.5 mg. with no thiazole), and in-

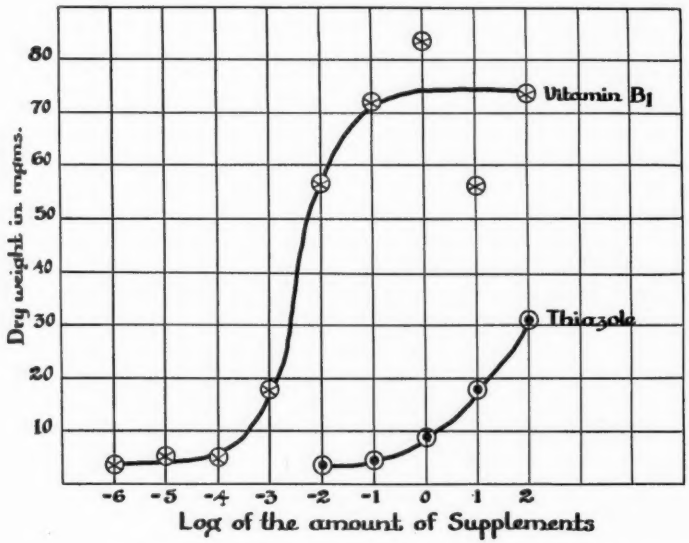


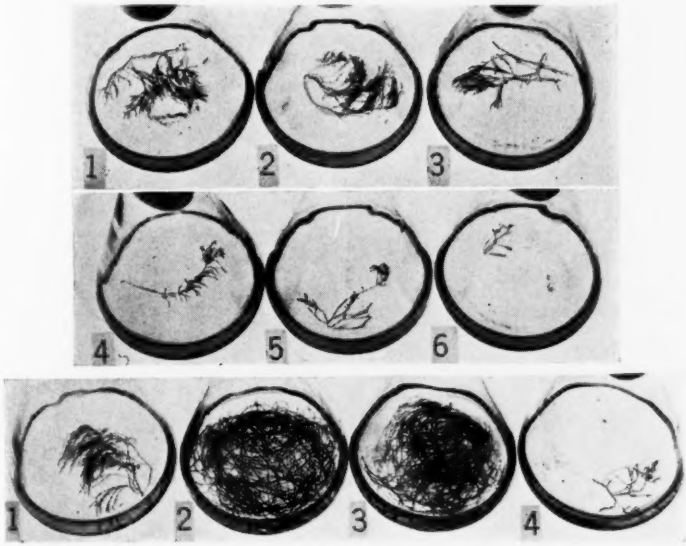
FIG. 11.—Relation between dry weights of individual roots and amount of vitamin B_1 or vitamin thiazole supplied. Grown in WHITE'S solution with yeast and cane sugar replaced by light brown commercial cane sugar. Logarithms of amount of supplement plotted against dry weight in mg.

creased growth occurred in the solutions containing up to 100 gamma.

Some solutions were included in passage 20, in which the sugar medium was supplemented with 10 gamma each of thiazole and bromopyrimidine. The roots grown in these solutions were indistinguishable from those grown with 10 gamma of vitamin B_1; the average dry weight of four roots was 69.4 mg., which is within the

range obtained with the higher concentrations of vitamin B_1 in the same medium (table 11). The similarity of the roots in the medium containing both thiazole and bromopyrimidine and those in the solution containing vitamin B_1 is shown in figure 13.

When various dilutions of mixtures of thiazole and pyrimidine were used, however, growth was not so good in the more dilute solu-



FIGS. 12, 13.—Fig. 12 (above), effect of various amounts of vitamin thiazole. Roots grown in WHITE'S solution with yeast and cane sugar replaced by light brown commercial sugar plus: *1*, 100 gamma thiazole; *2*, 10 gamma; *3*, 1.0; *4*, 0.1; *5*, 0.01; *6*, none. Fig. 13 (below), effect of vitamin B_1 and intermediates on growth of roots grown in WHITE'S solution with yeast and cane sugar replaced by light brown commercial sugar plus: *1*, 10 gamma vitamin thiazole; *2*, same plus 10 gamma vitamin pyrimidine; *3*, 10 gamma vitamin B_1; *4*, 10 gamma vitamin pyrimidine.

tions containing the intermediates as in those containing the vitamin.

In passage 21, begun June 9, 1937, a series of cultures were made in various concentrations of vitamin B_1, of thiazole, of pyrimidine, and of a mixture of thiazole and pyrimidine. The basic medium was WHITE'S mineral solution with 2% light brown sugar treated with

charcoal. Inoculum came from roots grown in WHITE's solution with Eastman's maltose replacing sucrose. The pieces of inoculum were rinsed in sterile mineral solution before they were placed in the culture flasks in order to remove the last traces of the medium in which they had grown.

On July 9, after 1 month of growth, roots in the mixture of thiazole and pyrimidine at the higher concentrations (10, 1.0, and

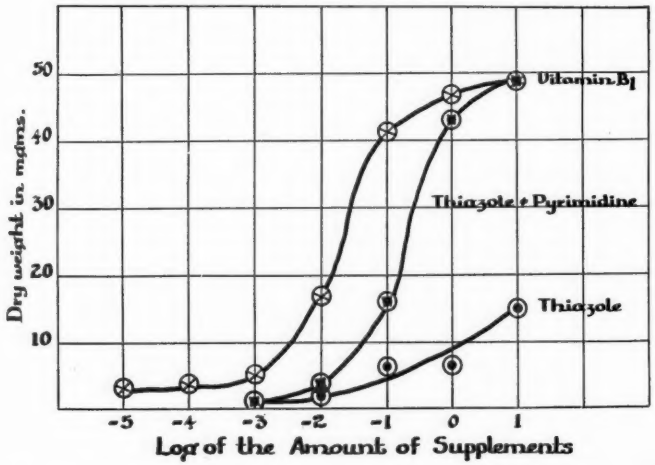


FIG. 14.—Effect of vitamin B_1, thiazole, and mixtures of thiazole and pyrimidine. Roots grown in WHITE's solution with yeast and cane sugar replaced by light brown sugar treated with charcoal. Logarithm of amount of supplements plotted against dry weight of roots in mg.

0.1 gamma per flask) resembled the roots in similar solutions containing the same concentrations of vitamin B_1; but a very decided difference was observed in the lower concentrations. This difference was obvious when the experiment was terminated in October, as shown by the curves in figure 14 and the data on dry weights in table 12.

In passage 21, the effect of 0.0001 gamma of vitamin B_1 was evident and the maximum growth was secured in the solutions containing 0.1 gamma or more. The charcoal evidently removed some beneficial material from the sugar, as the maximum growth in the

solutions containing the treated sugar (about 48 mg.) was not so great as in those which contained the untreated sugar (about 75 mg.). The results with different amounts of thiazole were similar to those secured with the untreated sugar in passage 20, except for those obtained with 0.0001 gamma of thiazole (table 12). No explanation other than experimental error can be offered for the aberrant results with this amount of thiazole.

TABLE 12

AVERAGE DRY WEIGHTS OF ROOTS FROM PASSAGE 21 IN WHITE'S MINERAL SOLUTION AND 2% LIGHT BROWN SUGAR TREATED WITH CHARCOAL AND SUPPLEMENTED WITH VARIOUS AMOUNTS OF VITAMIN B_1 OR ITS INTERMEDIATES

Amount supplied in gamma per flask	Vitamin B_1		Thiazole		Thiazole and pyrimidine		Pyrimidine	
	No. roots weighed	Dry weight per root (mg.)	No. roots weighed	Dry weight per root (mg.)	No. roots weighed	Dry weight per root (mg.)	No. roots weighed	Dry weight per root (mg.)
10.0	5	48.8	4	14.8	5	48.2	3	1.1
1.0	4	46.9	5	6.7	3	43.2	5	1.1
0.1	5	41.7	4	6.4	5	16.0	5	1.1
0.01	4	17.5	4	1.5	4	2.9	3	0.9
0.001	5	5.1	5	0.8	5	1.0	4	1.0
0.0001	5	3.8	4	3.2	4	0.3	4	1.0
None	3	0.7	3	0.7	3	0.7	3	0.7

No growth occurred with any of the amounts of pyrimidine used, except for two roots. One root in the medium composed of WHITE's mineral solution, 2% light brown sugar treated with charcoal, and 0.01 gamma of pyrimidine per flask grew as well as the roots with the same amount of vitamin B_1 in the same medium. The root in the solution supplemented with 0.01 gamma of pyrimidine weighed 17.4 mg. and the average for the roots in the medium with the same concentration of vitamin B_1 was 17.5 mg. The other root, grown in the same medium with 0.001 gamma of pyrimidine in the flask, weighed 4.5 mg.; and the average for roots in the solution with vitamin B_1 at the same concentration was 5.1 mg. When sketches of the inocula were examined, no difference was found which could explain the re-

sults, although this did not exclude the possibility that the particular pieces used might have differed in other respects than in appearance.

It was suggested that certain pieces may have been in a state of active growth, and having once started well, were able to grow in an unfavorable medium. To test this the following experiment was performed. In passage 22, roots from WHITE's solution with Eastman's maltose replacing sucrose were transferred to a medium composed of WHITE's mineral solution and 2% light brown sugar treated with charcoal, supplemented by vitamin B_1 at 0.0001 or 0.000001 gamma per flask. These concentrations of the vitamin had been found to produce some growth (table 11). The roots were allowed to grow for 2 weeks; then pyrimidine was added at the concentrations which had given growth in passage 21, namely, 0.01 or 0.001 gamma per flask. If our assumptions were correct, growth should have continued after the pyrimidine was added. The pyrimidine did not influence growth. No explanation other than experimental error can be offered for the growth of the two roots in the pyrimidine cultures.

The experiments described were performed with an obviously impure material, light brown sugar. What is the effect of dilution of the vitamin in a solution prepared with pure chemicals? The experiments with pure chemicals were not so elaborate as those with the light brown sugar medium. They showed, however, that amounts of vitamin B_1 as low as 0.0001 gamma affected the growth of the roots favorably.

In passage 15, begun November 24, 1936, besides subculturing roots in WHITE's solution with 10 gamma of B_1 replacing the yeast, four other concentrations were tested: 50, 1, 0.01, and 0.001 gamma per flask containing 50 ml. of medium. On December 6, after 10 days in the culture solutions, it was noted that roots were growing at each concentration (table 13).

In a second experiment (passage 16) roots were grown in WHITE's solution with yeast replaced by 10, 1, 0.01, 0.001, or 0.0001 gamma of vitamin B_1 per flask. A small but definite effect of 0.0001 gamma of the vitamin was noted by the appearance of the roots and in the dry weights.

The maximum growth in WHITE's solution with vitamin B_1 in-

stead of yeast did not equal that secured in the solutions in which the light brown sugar was used. In figure 15 the effect is shown of different amounts of vitamin B_1 in WHITE's solution lacking yeast, and in WHITE's solution with cane sugar and yeast replaced by vitamin B_1 and light brown sugar, or light brown sugar treated with charcoal. From these curves it is clear that something other than the amount of vitamin B_1 limited growth in WHITE's solution (lacking yeast) at the higher concentrations of the vitamin. We are inclined to believe that the limiting factor in WHITE's solution (with

TABLE 13

DRY WEIGHTS OF ROOTS FROM PASSAGE 15 GROWN IN WHITE'S SOLUTION AND IN SAME WITH YEAST EXTRACT REPLACED BY VARIOUS CONCENTRATIONS OF VITAMIN B_1

ADDITIONS TO WHITE'S SOLUTION WITHOUT YEAST	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
50 gamma vitamin B_1 per flask	9	8.2	1.7–12.0
1 gamma vitamin B_1 per flask	8	8.8	7.5–10.1
0.01 gamma vitamin B_1 per flask	6	3.5	1.1– 6.9
0.001 gamma vitamin B_1 per flask	8	7.2	1.7–10.2
Yeast extract 100 p.p.m.	5	7.8	3.3–10.4

vitamin B_1 instead of yeast) was probably the nature or amounts of mineral salts.

Although we have noted an effect of 0.000001 gamma of vitamin B_1 in the brown sugar medium, and of 0.0001 gamma in WHITE's solution lacking yeast, this does not imply that continued growth would occur in media containing so small an amount of the vitamin. In fact, in these experiments tomato roots ceased to grow when carried through successive passages in solutions containing 0.001 gamma or less of vitamin B_1. We have not determined the minimum amount of the vitamin necessary for unlimited growth of excised tomato roots. It is probably in the vicinity of 0.1 gamma per flask under the conditions of the experiments. It would be influenced by the duration of the individual passages, which would determine in part the total amount of vitamin available to a particular excised root in a given period of time.

It may be said, therefore, that while growth occurred with very low concentrations of vitamin B_1, solutions containing less than 0.001 gamma were not adequate to maintain the roots through successive passages. A concentration of 10 gamma of vitamin B_1 per flask, or 0.2 gamma per ml., was sufficient to maintain growth through thirteen passages extending over a period of 14 months.

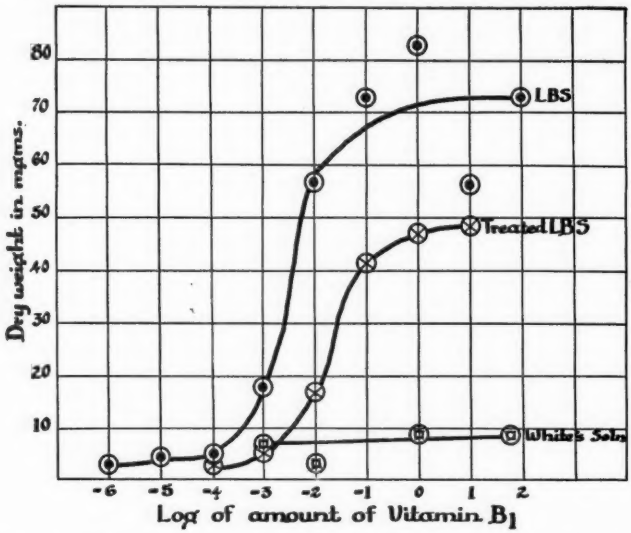


FIG. 15.—Effect of vitamin B_1 in different solutions. Logarithm of amount of vitamin B_1 plotted against dry weight of roots in mg. Below, WHITE's solution with yeast replaced by vitamin B_1. Above, WHITE's solution with yeast and cane sugar replaced by vitamin B_1 and light brown sugar. Middle, WHITE's solution with yeast and cane sugar replaced by vitamin B_1 and light brown sugar treated with charcoal.

CARBON SOURCES FOR EXCISED TOMATO ROOTS.—WHITE's solution contains sucrose as the source of carbon. Can tomato roots use carbon sources other than sucrose, and what is their relative value? We have investigated dextrose, levulose, maltose, mannose, cellobiose, inositol, pyruvic acid, and several samples of commercial cane sugar.[4] Dextrose and maltose proved satisfactory substitutes for cane sugar. Some growth was secured with levulose and cellobiose,

[4] All carbon sources were used at 2% concentration unless otherwise stated.

but none with the samples of mannose, inositol, or pyruvic acid used.

A. DEXTROSE AND MALTOSE.—In passage 11, seven pieces of inoculum were transferred to WHITE's solution containing Merck maltose, purified, no. 30504, instead of cane sugar, and seven to WHITE's medium with dextrose, Pfanstiehl's c.p. d-glucose no. 380, replacing the sucrose. The roots grew well in the solutions containing maltose or dextrose, but showed a characteristic type of growth different from that of those grown in WHITE's solution with sucrose. Excised roots in the sucrose cultures had short branches, many tips were swollen, and growth did not continue for much more than 1 month. In WHITE's solution with dextrose replacing sucrose the increase in length of branches was rapid, resulting in longer branches; the roots were more slender and were translucent rather than yellowish white. When examined with a hand lens, the roots in the dextrose solution showed noticeably fewer root hairs than did those in WHITE's solution with sucrose. A swollen condition of the tips was sometimes noticeable after $1\frac{1}{2}$ to 2 months in the dextrose cultures, but it was not so pronounced as in the WHITE's solution with sucrose. The type of growth in the maltose cultures was different from that in either the dextrose or sucrose solutions. The branches were long, slender, and very white; root hairs were more numerous than in the dextrose cultures. Swollen tips were rare in the maltose cultures; and growth continued for more than 2 months. Roots grown with these three sugars are shown in figure 16. It was not easy to determine when growth in the maltose solutions actually stopped, for the roots appeared white and the tips were normal when examined after 5 months of growth.

Subcultures of the roots in dextrose and in maltose were made to the same solutions for a number of successive passages (table 14). The same types of growth were obtained throughout the experiments with maltose and dextrose.

From the experiments it appeared that excised tomato roots were able to utilize dextrose, although WHITE (30) has reported otherwise. We have carried roots through six successive passages (table 14) in WHITE's solution with sucrose replaced by dextrose. In fact, so far as dry weights are concerned, the dextrose was at least as

satisfactory as cane sugar. Two samples of dextrose were used, Pfanstiehl's c.p. d-glucose and cerelose (in one passage only). The temperature of sterilization (110° or 120° C.) of the dextrose had little effect upon growth of the roots (passage 14, table 14). Growth occurred also in dextrose solutions which were filtered sterile. It

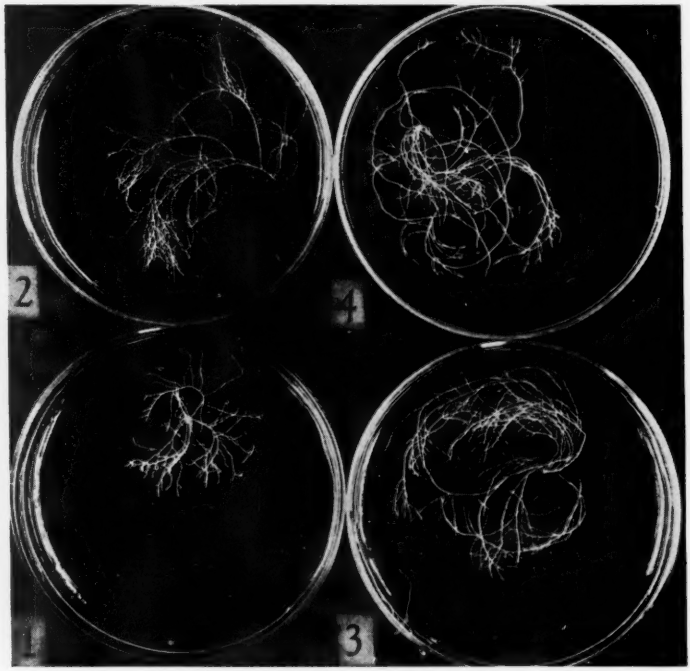


FIG. 16.—Various carbohydrates and growth of tomato roots: *1*, WHITE'S solution (cane sugar); *2*, cane sugar replaced by dextrose; *3*, by maltose; *4*, cane sugar and yeast replaced by maltose.

was found further that subcultures of the clone of excised tomato roots used by WHITE[5] grew in a dextrose solution. These results have been discussed elsewhere (**20**).

We are not certain whether the difference between the character of the growth noted in media containing dextrose and that in those

[5] These subcultures were furnished through the courtesy of Dr. P. R. WHITE.

containing cane sugar was associated with the carbohydrate, or with the traces of contaminants present in these purified sugars. WHITE's solution can be improved by the addition of mineral supplements (see discussion of mineral nutrients later), and all of the carbohydrates used contain traces of many elements. For example, al-

TABLE 14

DRY WEIGHTS OF ROOTS GROWN THROUGH SUCCESSIVE PASSAGES IN (A) WHITE'S SOLUTION WITH SUCROSE REPLACED BY DEXTROSE; (B) SAME WITH SUCROSE REPLACED BY MERCK'S MALTOSE NO. 30504; (C) WHITE'S SOLUTION. IN SOLUTION A THE DEXTROSE WAS PFANSTIEHL'S DEXTROSE NO. 380; IN A′, CERELOSE

PASSAGE	CULTURE SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
11	A	5	25.7	17.5–30.0
	B	5	62.4	54.7–72.4
	C	7	14.8	7.0–19.5
12	A	4	10.5	6.3–12.6
	B	5	25.5	19.4–26.7
	C	6	22.3	17.0–29.2
13	A	4	11.6	9.7–14.5
	B	4	14.4	5.8–20.8
	C	6	8.5	4.5–11.0
14*	A-110° C.	7	12.2	1.7–21.4
	A-120° C.	7	13.1	4.5–24.6
	B-110° C.	8	10.4	0.2–33.8
	B-120° C.	5	12.1	2.4–18.7
	C-110° C.	9	6.7	4.2–12.1
	C-120° C.	6	8.0	6.6–10.5
15	A	7	25.4	18.1–29.6
	C	5	7.8	3.3–10.4
16	A	9	4.5	1.1–11.4
	A′	7	7.1	1.8–10.4
	C	28	5.4	0.9–10.4

* Some solutions sterilized at 110° C. for 30 minutes and others at 120° C. for 20 minutes.

though our sample of cerelose had only 0.004% ash, it contained traces of twenty elements, as shown by spectrographic analysis. Qualitative spectographic analyses were made by arcing the ash of cerelose (dextrose) directly on graphite electrodes by A. P. VANSELOW through the courtesy of Dr. W. P. KELLEY of the University

of California. Elements present in large amounts were Ca and Na; in definite traces, Ag, Al, Ba, Cr, Cu, Fe, K, Mg, Ni, Pb, and Sr; and elements just detectable, B, Mn, Pt, Si, Sn, Ti, and Zn. Although an analysis of the ash of other carbon sources was not made, all the samples used contained minerals in varying amounts, as shown in table 15.

In these experiments the effect of any carbon source, even its apparent availability, was found to depend to a considerable extent on the particular sample used. This was true even with the purified

TABLE 15

ASH CONTENTS OF SUGARS USED AS CARBON SOURCES

CARBOHYDRATE	SAMPLE	PERCENTAGE ASH
Dextrose	Pfanstiehl no. 380	0.008
	Cerelose	0.004
Maltose	Pfanstiehl no. 602	0.358
	Merck no. 30516	0.092
	Eastman	0.138
Sucrose	Pfanstiehl no. 409	>0.00001
	Light brown	1.909
	Dark brown	3.380

samples. These statements may be illustrated by the results with maltose. Merck's purified maltose no. 30504 proved entirely satisfactory as a source of carbon for excised tomato roots. In four successive passages (table 14) the growth in solutions containing this maltose was superior to that in the solutions containing cane sugar or dextrose. However, Pfanstiehl's c.p. maltose no. 602 was decidedly injurious; no growth was obtained in several trials with this sugar replacing sucrose in WHITE's solution. A sample of Eastman's maltose was almost as satisfactory as Merck no. 30504. Growth with Merck purified maltose no. 30516 was poor; with Merck purified maltose no. 22068 the growth was better, but not so good as with the Eastman maltose. Depending upon the sample used, it would be possible to conclude that maltose was not available to tomato roots, that maltose was better than cane sugar, or that maltose was poorer than cane sugar as a source of carbon.

It is noteworthy, however, that in each instance where growth occurred in the maltose solutions the roots had the same general appearance, irrespective of the sample of maltose used. They were white in color, had normal appearing tips on their branches, and produced relatively long branches, not short and stubby ones as were frequent in the sucrose solutions.

In addition to the experiments in which different samples of maltose were used to replace sucrose in WHITE's solution, a number of the same samples of maltose, which had been found to be satisfactory carbon sources, were used to replace both sucrose and the yeast extract in WHITE's medium. In general the samples which had proved most satisfactory as carbon sources were best when used in place of both sugar and yeast.

B. LEVULOSE AND CELLOBIOSE.—In passage 16, subcultures were made in WHITE's solution with cane sugar replaced by levulose or by cellobiose. Both compounds came from the Eastman Kodak Co. The amount of growth in the levulose solutions was almost as great as in WHITE's solution, but was considerably less in the cellobiose solutions. No subcultures were made in solutions containing these two carbon sources, and only the one sample of each was tested. The type of growth in the two media was similar to that obtained with dextrose: long, slender, translucent roots, with few root hairs.

C. MANNOSE, PYRUVIC ACID, AND INOSITOL.—In passage 19, mannose and pyruvic acid were tested as possible carbon sources for excised tomato roots. The mannose was Pfanstiehl's sample no. 587, and the pyruvic acid was an Eastman product. Mannose was used at 2% concentration; and pyruvic acid, adjusted to pH 5.0, was used at two concentrations, 0.5 and 0.1%. No growth was obtained with either of these carbon sources. Other samples of these compounds were not at hand, and no final conclusion as to availability of mannose and pyruvic acid can be drawn.

In passage 22, a sample of inositol, an Eastman product, was used in place of sucrose in solution W plus vitamin B_1. No growth was obtained with this sample of inositol.

D. COMMERCIAL CANE SUGARS.—In addition to the pure (Pfanstiehl c.p.) cane sugar used in WHITE's solution, four samples of commercial cane sugar have been used: a dark brown sugar, two

light brown sugars, and a white granulated sugar. The dark brown sugar was injurious. No growth occurred in WHITE'S solution with the pure cane sugar replaced by the dark brown sugar. The white granulated sugar also appeared to be somewhat injurious. Poorer growth was secured in solution Z containing this sugar and yeast than in the same solution containing the "chemically pure" sugar. However, the medium containing the light brown sugars proved

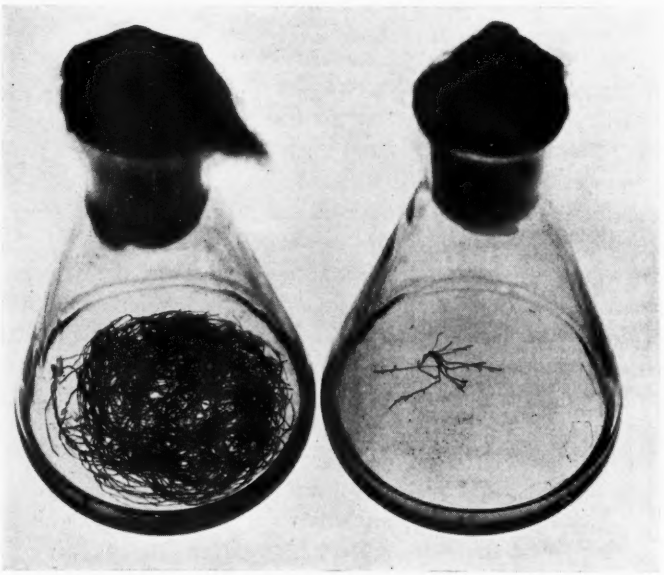


FIG. 17.—Effect of vitamin B_1. WHITE'S solution with yeast and cane sugar replaced by light brown sugar: left, plus 10 gamma vitamin B_1 (dry wt. 80.0 mg.); right, no vitamin B_1 (dry wt. 1.0 mg.).

superior to any medium we have used. In fact, so excellent was the growth with this impure sugar that it was used in a number of our experiments with vitamin B_1 or intermediates (fig. 17).

The only other medium which gave growth comparable with that obtained in the light brown sugar medium was one composed of WHITE'S minerals, "chemically pure" sucrose, and a water extract of malt flour. The malt flour cultures in which fresh malt flour ex-

tract was added four times at intervals of 2 weeks gave growth within the range of that obtained with light brown sugar.

The largest root (dry weight 91.3 mg.) obtained in our experiments was grown in a medium composed of WHITE's mineral solution, 2% light brown sugar, and 10 gamma of vitamin B_1 per flask. The root was grown at room temperature and remained in the flask from March 5, 1937 until October 20, 1937. It is not known whether the root was still growing when the experiments were terminated, for measurements at intervals were not possible; however, many of the branches were white at the tips and appeared normal at the end of the experiments.

We are not prepared to explain the highly beneficial effect of the light brown sugar. It was evidently not because of its content of vitamin B_1 nor of thiazole. Very little growth was secured in solutions containing this sugar unless vitamin B_1 or thiazole was also present. The ash of the sugar used in amounts equivalent to that in a 2% solution of the sugar proved toxic. It is possible, however, that smaller amounts of the ash might be beneficial. Part of the beneficial material was removed by treatment with Merck medicinal charcoal or with Fuller's earth. The ash content (1.9%) of this sugar was high and its beneficial action may be associated with the minerals it contains. Our experiments, however, have not eliminated the possibility that organic material other than sucrose, vitamin B_1, or thiazole may be concerned.

MINERAL NUTRIENTS.—The mineral salts in WHITE's solution are supplied by a known mixture of the composition given earlier. In addition there is an unknown mixture of minerals added in the yeast extract. The concentration of salts from the known mixture in WHITE's solution was about 375 p.p.m. From the ash of a water extract of yeast it is estimated that about 5 p.p.m. of mineral material was added with the yeast extract used in WHITE's solution. Are the minerals in the yeast extract of significance in determining growth in WHITE's solution? Is the known mixture of salts in WHITE's solution adequate for the best growth of excised tomato roots? Can some other mixture be substituted for it?

In our experiments the ash elements added with the yeast appeared to be of some importance. The growth in WHITE's solution

in which the yeast was replaced by vitamin B_1 and yeast ash was superior to that in the same solution in which the yeast was replaced by vitamin B_1 alone. In fact the vitamin and yeast ash together gave results somewhat better than the yeast.

Some attempt was made to determine what specific mineral substances were effective in the yeast ash. Since the yeast was added to a solution which already contained the common essential mineral elements, our attention was directed toward the rarer elements. The effects of boron, zinc, and a modification of Hoagland's A to Z mix-

TABLE 16

DRY WEIGHTS OF ROOTS FROM PASSAGE 18 GROWN IN WHITE'S SOLUTION AND THE SAME WITH YEAST REPLACED BY VITAMIN B_1 ALONE OR BY VITAMIN B_1 AND MINERAL SUPPLEMENTS

SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
White's solution with vitamin replacing yeast	9	9.2	2.6–11.2
Solution W plus vitamin B_1	10	12.2	0.4–20.3
Solution Z plus vitamin B_1	10	9.3	0.3–39.9
White's solution	14	14.1	7.7–23.4

ture were tested (fig. 20). When the yeast in WHITE'S solution was replaced by vitamin B_1, 0.1 p.p.m. of B (as boric acid), and 0.1 p.p.m. of Zn (as zinc sulphate), the growth was almost as good as in WHITE'S solution and superior to that in WHITE'S solution with yeast replaced by vitamin B_1 alone (table 16). It would seem, therefore, that a part of the beneficial action of the yeast extract is related to its boron and zinc content.

Results with the modification of Hoagland's A to Z mixture were irregular. In some experiments a decidedly favorable effect was observed, in others the mixture appeared to be somewhat injurious. It is possible that a dilution of the mixture would be preferable.

While the mixture of salts in solution W was superior to the known mixture in WHITE'S solution, it is probable that a further improvement could be made. This is suggested by the fact that growth in WHITE'S solution was somewhat superior to that in the same

medium with yeast replaced by vitamin B_1, boron, and zinc and because some samples of filter paper or of filter paper ash improved growth when added to WHITE's solution in which yeast was replaced by vitamin B_1, boron, and zinc.

In passage 6, WHITE's solution was supplemented by adding ½ sheet of a sample of A. H. Thomas qualitative filter paper to each flask. ROBBINS and WHITE (16) had found this filter paper beneficial to excised corn roots. It was also effective as a supplement to WHITE's solution for tomato roots. Excised roots of tomato grown

TABLE 17

DRY WEIGHTS OF ROOTS GROWN IN WHITE'S SOLUTION AND IN SAME SOLUTION SUPPLEMENTED BY TWO SAMPLES OF FILTER PAPER

PASSAGE	ADDITIONS TO WHITE'S SOLUTION	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
6.....	None	6	14.6	10.0–17.5
	Filter paper	9	46.7	32.0–71.0
18.....	None	14	14.1	7.7–23.4
	Filter paper	6	20.3	11.2–32.2

in WHITE's solution containing filter paper had much longer branches and were whiter in color than those in WHITE's solution (fig. 18). In passage 18 another sample of A. H. Thomas qualitative filter paper was used. Again beneficial results were obtained; but they were not so marked as were those in the earlier experiment (table 17). A third sample of filter paper completely inhibited growth.

If filter paper added something to WHITE's solution which made it more suitable for growth, was it mineral in character? To test this, filter paper ash was used as a supplement. The results varied with the sample of filter paper used and the treatment of the ash. The ash of some samples was highly beneficial, especially when treated with hydrochloric acid; that of others was injurious. In passage 15 filter paper ash was used in WHITE's solution with vitamin B_1 replacing the yeast. Ash of ½ sheet of filter paper per flask was used, but practically no growth was obtained (table 18). In a later experiment, passage 22, the same sample of filter paper ash was

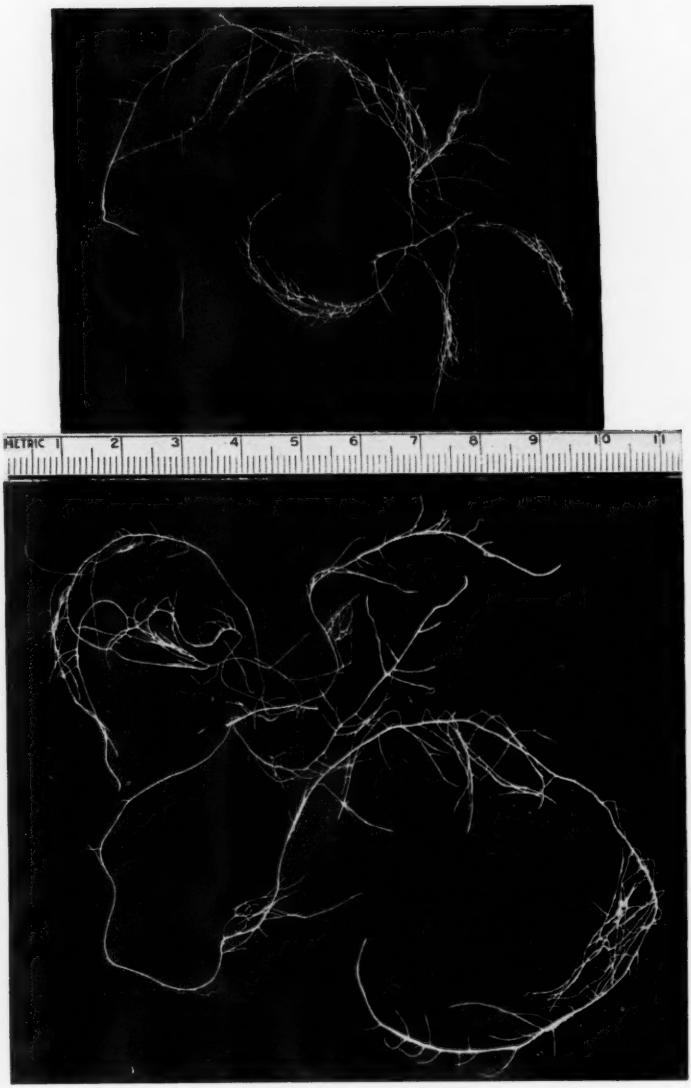


FIG. 18.—Effect of filter paper (passage 6): above, excised root in WHITE's solution; below, same plus ½ sheet filter paper.

treated with hydrochloric acid, the acid boiled off, and the residue used in solution W plus vitamin B_1. In this experiment the filter paper ash proved highly beneficial (table 18). Two out of three roots weighed were much superior to any of those in solution W with vitamin B_1 alone in this and other experiments. The best roots from the two sets are shown in figure 19.

TABLE 18

DRY WEIGHTS OF ROOTS SHOWING EFFECTS OF ASH OF FILTER PAPER AND OF SAME ASH TREATED WITH HYDROCHLORIC ACID

PASSAGE	ADDITIONS TO WHITE'S SOLUTION WITH VITAMIN B_1 REPLACING YEAST	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
15	None	7	7.1	2.7–17.1
	Filter paper ash (untreated)	9	0.5	0.3– 0.9
22	Boron and zinc 0.1 p.p.m.	6	7.5	5.2– 8.2
	Boron and zinc plus filter paper ash treated with HCl	3	23.6	3.0–45.5

Are the concentrations and actions of salts used in the known mixture in WHITE's solution more satisfactory than other solutions? We have used two other basic mineral solutions:

Solution C (16), with the following composition:

$Ca(NO_3)_2$	0.25 gm.
$MgSO_4$	0.05
KH_2PO_4	0.05
$FeCl_3$	0.005
H_2O	5000.00 ml.

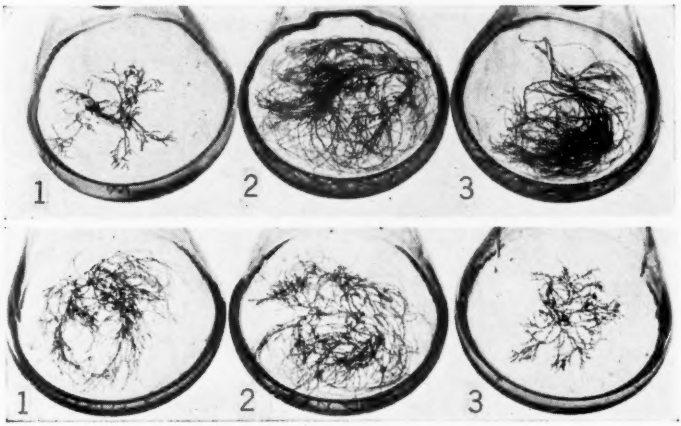
A modified Pfeffer's solution (10):

$Ca(NO_3)_2$	2.0 gm.
KH_2PO_4	0.5
KNO_3	0.5
KCl	0.25
$MgSO_4$	0.5
$FeCl_3$	0.005
H_2O	6000.00 ml.

No appreciable growth was secured when either of these mineral solutions was used with the same concentration of yeast and cane sugar as is present in WHITE's solution. When the $FeCl_3$ in Pfeffer's

solution was replaced by 2.5 p.p.m. $Fe_2(SO_4)_3$, however, growth occurred although not to so great an extent as in WHITE's solution.

The modified Pfeffer's solution with sucrose and water extract of yeast and 2.5 p.p.m. $Fe_2(SO_4)_3$ was not so satisfactory as WHITE's solution. However, the modified Pfeffer's solution with 2.5 p.p.m. of $Fe_2(SO_4)_3$ plus 0.1 p.p.m. of B and 0.1 p.p.m. of Zn proved superior to solution W when each solution was supplemented with vitamin B_1.



FIGS. 19, 20.—Fig. 19 (above), effect of filter paper ash (passage 22). Roots grown in: *1*, solution W plus vitamin B_1; *2*, same plus filter paper ash treated with HCl; *3*, WHITE's solution with Eastman's maltose instead of cane sugar. Fig. 20 (below), effect of mineral supplements. Roots grown in WHITE's solution with yeast replaced by vitamin B_1: *1*, plus 0.1 p.p.m. boron and zinc; *2*, plus A to Z mixture; *3*, no addition.

In passage 22, excised tomato roots were grown in: (a) WHITE's mineral solution; (b) modified Pfeffer's solution; (c) modified Pfeffer's solution with 2.5 p.p.m. of $Fe_2(SO_4)_3$; (d) modified Pfeffer's solution with 3.2 p.p.m. $FeCl_3$. To each solution 2% cane sugar, 0.1 p.p.m. B, 0.1 p.p.m. Zn, and vitamin B_1 were added. In passage 23, subcultures of roots grown in these four solutions were made to the same media. Results of this experiment are summarized in table 19.

The Pfeffer's solution containing 0.1 p.p.m. of B and Zn, 2% cane sugar, and vitamin B_1 was not satisfactory (table 19), but when the

0.8 p.p.m. of $FeCl_3$ in this solution was replaced by 2.5 p.p.m. of $Fe_2(SO_4)_3$ it proved superior to WHITE's mineral solution containing the B, Zn, cane sugar, and vitamin B_1. When the $Fe_2(SO_4)_3$ in the Pfeffer's solution was replaced by $FeCl_3$, containing an equivalent amount of Fe, the results were about the same as those secured with WHITE's mineral solution, but decidedly inferior to those with the Pfeffer's solution containing 2.5 p.p.m. $Fe_2(SO_4)_3$.

TABLE 19

DRY WEIGHTS OF ROOTS THROUGH TWO PASSAGES IN MEDIUM COMPOSED OF MINERAL SOLUTION PLUS BORON AND ZINC 0.1 P.P.M., 2% SUCROSE AND VITAMIN B_1: (A) WHITE'S MINERAL SOLUTION; (B) MODIFIED PFEFFER'S SOLUTION; (C) SAME WITH 2.5 P.P.M. $Fe_2(SO_4)_3$ REPLACING THE $FeCl_3$; (D) SAME WITH 3.2 P.P.M. $FeCl_3$ REPLACING THE 0.8 P.P.M. $FeCl_3$

PASSAGE	MINERAL SOLUTION USED	NO. ROOTS WEIGHED	DRY WEIGHT PER ROOT (MG.)	RANGE DRY WEIGHT (MG.)
22	A	5	4.9	0.6– 9.7
	B	4	2.9	2.5– 3.2
	C	4	20.1	16.6–22.3
	D	3	6.2	4.0– 8.5
23	A	7	5.6	0.4– 9.6
	B	4	1.6	0.9– 2.1
	C	4	12.1	10.5–15.5
	D	4	4.2	3.7– 5.5

Why should the $Fe_2(SO_4)_3$ be superior to the $FeCl_3$? Possibly not because of the Fe content but more probably because of a greater amount of contaminants in the $Fe_2(SO_4)_3$, perhaps manganese. Analyses of the $FeCl_3$ by the periodate method showed the presence of 0.0013% Mn. The $Fe_2(SO_4)_3$ contained approximately forty times this amount of Mn.

SOURCES OF NITROGEN.—WHITE's solution contains $Ca(NO_3)_2$ and KNO_3. In addition, amino acids and other types of nitrogenous compounds are supplied in the extract of yeast. Is the organic nitrogen in WHITE's solution important as a source of nitrogen? Can excised tomato roots utilize nitrates as a source of nitrogen?

In our experiments excised tomato roots appeared to be capable of utilizing nitrates; in fact, they required no other source of nitro-

gen. This conclusion followed because the roots grew through fourteen passages over a period of more than 14 months in a solution of mineral salts, pure cane sugar, and synthetic vitamin B_1. When the nitrates were omitted from this solution no growth occurred.

For the first 4 months WHITE's solution with the yeast replaced by 10 gamma of vitamin B_1 per flask was used and for the last 10 months solution W plus cane sugar and vitamin B_1. The nitrogen furnished by the vitamin in these solutions was 0.03 gamma N per ml., or 1.5 gamma per flask. The nitrates in WHITE's solution or in solution W furnished 28 gamma of N per ml., or 1400 gamma per flask.

To test the possibility that vitamin B_1, even at this low concentration, might furnish nitrogen for growth of the roots, solutions without nitrates were tried. In passage 22, root fragments were transferred to WHITE's solution with vitamin B_1 replacing the yeast, and to the same solution without nitrates. The $Ca(NO_3)_2$ and KNO_3 were replaced by $CaCl_2$ and KCl respectively. No growth was visible in the solution without nitrates.

In this same passage, ammonia was used in place of nitrates. Ammonium chloride, equivalent in nitrogen to that in the nitrates, was used in WHITE's solution without nitrates. No growth was obtained. In passage 24, both ammonium sulphate and ammonium chloride were used as substitutes for nitrates in WHITE's mineral solution; they were used at concentrations such that the nitrogen was one-tenth that in WHITE's solution. No growth occurred. As an additional check, ammonium chloride, at the same concentration as in passage 22, was added to solution W plus vitamin B_1. Growth, not quite so profuse as that obtained in solution W plus vitamin B_1 alone, took place. Evidently a failure of roots to grow in a solution with NH_4Cl as the only source of nitrogen was not related to the toxicity of the ammonium salt.

It is concluded that excised tomato roots required for growth no other source of nitrogen than nitrates, and that nitrogen in the form of ammonia was not available under the conditions of our experiments. It appears probable that unlimited growth of tomato roots would occur with no other source of nitrogen than nitrates.

Was the amount of nitrogen in WHITE's solution or in solution W

a limiting factor for the growth of tomato roots in our experiments? We believe it was not. The increased growth obtained on the addition of boron and zinc to the minerals of WHITE's solution would suggest that nitrogen was not a limiting factor in that solution. The increased growth obtained through the addition of filter paper ash to solution W containing vitamin B_1 would suggest the same conclusion. If we assume 2.5% N in the dry matter of these roots (**9**), then the N of the nitrates in 50 ml. of WHITE's solution should permit the formation of about 60 mg. of dry matter. The maximum dry weight secured in 50 ml. of WHITE's solution was usually between 20 and 30 mg. (in one instance 43 mg.). We are inclined to believe that growth was limited by accessory mineral elements present in inadequate amounts as contaminants in WHITE's solution. The limiting action of supplementary mineral elements was more marked when the yeast was replaced by vitamin B_1 because of the elimination of the supplementary elements in the yeast ash.

LIGHT AND DARKNESS.—Limited experiments with roots grown in the dark have confirmed WHITE's report (**31**) that light is unnecessary for continued growth of excised tomato roots. Roots grew in successive passages in the dark in WHITE's solution.

VARIABILITY IN GROWTH.—Throughout these experiments considerable variability was noted in the growth of excised tomato roots under similar conditions. One root might develop several times as much as another, even though both were grown in aliquots of the same solution for the same period of time and under the same conditions of light and temperature. The variability to which we refer is not that resulting from complete failure of a particular root fragment to grow, although we have included some such instances in the data. Even when both roots under consideration grew, one might develop much better than the other. Examples of this may be found by examining ranges in dry weights given in the tables.

ROBBINS and WHITE (**16**) were of the opinion that the somewhat similar results found with excised roots of corn might be because of its heterozygous condition. Such an explanation would not apply here. The excised tomato roots with which we have worked were from two clones, B and C, derived from two original roots. The variability referred to was noted in roots from each clone; further-

more no consistent differences were noted between the growth of roots from clone B or clone C under the same conditions.

We do not believe that the variability can be accounted for by differences in the solutions, contamination of glassware, or the environmental conditions under which the roots were grown. Aliquots of the same solution were used in any particular experiment. The glassware was carefully cleansed with chromic acid cleansing mixture and rinsed six times with tap water, three times with distilled water, twice with redistilled water, and dried by draining. A given series of flasks were placed together under the same light and temperature conditions.

We do not believe that injury in fragmenting or transferring can account for the variability. Injury might account for the failure of an occasional fragment to grow, although care was always used to cool the knives and needles employed in fragmenting and handling the roots. Injury would not seem responsible where one piece grew poorly and another well.

The character of the root fragment used as inoculum may be responsible, although this has not been demonstrated. Variability in development of pieces which appeared similar at the time of inoculation was noted. But even though two pieces appear much alike, they may have come from different parts of a root and may differ in relative proportions of various tissues and in chemical composition.

It is not probable that the number or proportion of growing points was significant. This conclusion followed from results of an experiment in which the growth of pieces without evident growing points was compared with that of those with one or more growing points. Both types grew and no difference in the extent of growth was noted.

Evidence accumulated during these experiments that the nature of the solution in which the root fragmented for inoculum grew may influence its later development. This effect, which we have called carry-over, is discussed in the next section. The examples of carry-over noted were not sufficiently great to account for the variability observed. Furthermore the inoculum for any particular set was, as a rule, taken from roots which had developed in solutions of uniform composition, and frequently from a single root.

CARRY-OVER.—When roots which were growing rapidly were used as sources of inoculum for an unfavorable medium, considerable

growth often occurred in the first passage; but subcultures in the unfavorable medium failed to grow. This carry-over effect was noted in a number of experiments. For example, some growth occurred when WHITE'S solution without yeast was inoculated with rather large pieces of roots which had been growing in WHITE'S solution. No growth could be detected when this same solution without yeast was inoculated with a root fragment from a root grown in a solution containing 0.001 gamma or less of vitamin B_1 per flask.

In passage 20, flasks of a solution composed of mineral solution and light brown sugar treated with charcoal were inoculated from an Eastman's maltose culture. The pieces of inoculum were rinsed in sterile mineral solution. The roots grew appreciably in this medium, which contained no yeast or substitute for yeast. The average dry weight for four roots was 1.0 mg., and the largest root weighed was 1.5 mg.

Results showed that pieces of excised roots, even when rinsed in sterile mineral solution, carried with them enough vitamin B_1 or substitute to produce appreciable growth, but not enough to maintain this growth through more than one passage.

VITAMIN B_1 AND THIAZOLE AS RELATED TO SULPHUR.—Vitamin B_1, or its intermediate thiazole, was found to be essential for the growth of excised tomato roots. In addition to carbon, hydrogen, nitrogen, and oxygen, these compounds contain sulphur. Do they function as available sources of sulphur? To test this the roots were grown in WHITE'S solution containing vitamin B_1 or thiazole but without inorganic sulphur. The $MgSO_4$ in WHITE'S solution was replaced by $MgCl_2$, and the $Fe_2(SO_4)_3$ was replaced by $FeCl_3$. The roots in the solutions containing vitamin B_1 or thiazole but lacking inorganic sulphur failed to grow. From these experiments it was concluded that the vitamin B_1 or thiazole used in the culture media did not replace the sulphur in the mineral solution.

Discussion

Excised roots of a green plant, *Lycopersicon esculentum* Mill., were found to require for unlimited growth mineral salts, sugar, and thiazole (or vitamin B_1) in addition to water and dissolved gases. These investigations have not entirely eliminated the possibility that other sources of carbon might be substituted for sugar and

other compounds might substitute for vitamin B_1 or thiazole. Furthermore it is conceivable that the requirements of excised tomato roots in liquid culture may not be the same as those of roots attached to the tomato plant and growing under natural conditions in an aerated soil. Nevertheless it is reasonable to assume from the results that the tomato root requires an external supply of both sugar and thiazole (or vitamin B_1). If this assumption is correct, it would appear that the attached tomato root depends upon the top for both sugar and thiazole (or vitamin B_1). Data on the synthesis of sugar by the tomato top, its translocation to the root, and its utilization are sufficient to form a clear concept of this relation. Similar information on vitamin B_1 or thiazole is lacking.

Why should the top of the tomato plant form thiazole (or vitamin B_1) while the root does not? It would not appear to be a light relationship, since some fungi apparently form vitamin B_1 in the dark. It would not appear to be related necessarily to chlorophyll, since some fungi (which lack chlorophyll) form vitamin B_1 (**5**).

Where and how is thiazole translocated in the plant? It would be reasonable to assume that it is translocated through the phloem, although definitive evidence on this point is lacking. If it is translocated through the phloem, how significant in ringing or girdling experiments is interference with its movement? Experimenters in this field have hitherto confined themselves largely to a consideration of carbohydrates, nitrogen, and mineral salts.

Is thiazole, alone, necessary for the growth of the tomato root, or does the root actually require vitamin B_1, synthesizing pyrimidine but little or no thiazole and forming the vitamin when supplied with thiazole? We are inclined to believe that the latter assumption is correct.

If the vitamin is required, how does it function? On this we have little information. In his work with *Phycomyces*, SCHOPFER has suggested that vitamin B_1 functions in nitrogen metabolism (**24**). His evidence is not entirely convincing, but his hypothesis offers one possibility. Suggestions have been made that vitamin B_1 is concerned with carbohydrate metabolism in animals, and some support has been accumulated for this hypothesis (**27**).

The extremely minute amounts which are effective, and the huge

ratio of dry matter formed to vitamin present, are intriguing facts which must be considered in any hypothesis dealing with the function of vitamin B_1. These questions and many others are raised by our findings of the essential nature of thiazole (or vitamin B_1) in relation to the growth of the tomato root.

In any event, our results and those of BONNER (**1, 3**), secured independently with excised pea roots, confirm the original hypothesis of ROBBINS, and of ROBBINS and MANEVAL. This hypothesis was that the failure of excised roots to make unlimited growth in solutions of mineral salts and sugar is because of the lack in the medium of some necessary material derived by the seedling root from the grain and exhausted during growth of the root. WHITE's conclusion (**29**) that our original experiments which indicated the existence of a "hormonal" factor must be explained in some other way is in error. He neglected to consider the materials included in the yeast extract which was added to the medium.

As already pointed out, the root (which contains no chlorophyll) depends upon the green top for the carbohydrates used in its metabolism. It appears that tomato roots are not highly specific as to sources of carbon which can be utilized. They grew well with dextrose, levulose, maltose, sucrose, and to some extent with cellobiose. Our experiments, however, did not include a sufficient variety of carbon sources to permit us to conclude much on the availability of different kinds of carbon compounds and the way in which they are assimilated. It appears clear from our results, especially with different samples of maltose, that in such work greater care than is usual should be given to the purity of the compounds used.

These experiments show that excised tomato roots are capable of using nitrates in the synthesis of their proteins. This is significant since it indicates that root cells in the green plant may not necessarily depend upon the top for organic nitrogen. While excised tomato roots appear capable of synthesizing protein from nitrates, this does not exclude the possibility that an attached root may derive organic nitrogen from the top and be benefited thereby. WHITE (**33**) and BONNER and coworkers (**1, 2, 3**) have reported a beneficial action on the growth of excised roots from the addition of amino acids to a solution of mineral salts, cane sugar, and vitamin B_1. But we cannot

agree with BONNER and WHITE's statements that amino acids are essential or necessary for the growth of excised roots.

Since these cells are able to utilize nitrates, it might be anticipated that ammonia also would be available. Under the conditions of our experiments the excised roots were unable to grow with ammonium salts as the sole source of nitrogen. Could this failure be related to a deficiency in oxygen? It is possible that nitrates may be important in the respiration of tomato roots submerged in liquid cultures. No experiments which would clarify this question have been performed.

The synthetic media, composed of mineral solution, pure sucrose, and vitamin B_1 or thiazole, have proved sufficient for growth through a number of passages with no consistent lessening of growth. The growth of excised roots is not so rapid in these media, nor does it continue so long in a single passage as it does in certain other non-synthetic media, such as those containing light brown sugar or malt flour. The conclusion might be drawn that the synthetic media were not adequate, because better growth occurred in other solutions. Everything necessary for continued growth was supplied by these synthetic solutions, however, and there is little reason to doubt that growth comparable in amount to that obtained in the "better" media could be obtained if fresh supplies of the synthetic media were furnished at frequent intervals.

In spite of the high purity of the cane sugar used in our experiments, a purity indicated by an ash content of less than 0.0001%, it must be remembered that cane sugar is a natural product and may contain traces of organic impurities. Although we believe a solution of mineral salts, sugar, and vitamin B_1 or thiazole is adequate for unlimited growth of excised tomato roots, a complete demonstration must wait experiments in which some synthetic carbon compound is successfully substituted for those derived from natural sources.

More attention should be devoted to the rarer mineral elements as limiting factors in the growth of excised roots. It is likely that the superiority of $Fe_2(SO_4)_3$ over $FeCl_3$ is not associated with the Fe, as believed by WHITE (**28**), but with the mineral contaminants present to greater degree in the $Fe_2(SO_4)_3$. Also, the factor limiting growth of excised tomato roots in WHITE's solution may be the amount of one or more of the rarer elements. It is unfortunate that supplies of high-

ly purified chemicals are not available commercially for physiological work. Until they are, and until there is a more general realization of the importance of traces, conclusions on the mineral nutrition of excised roots will be faulty.

Summary

1. The possibility of unlimited growth of excised tomato roots in WHITE's solution composed of mineral salts, sugar, and yeast extract was confirmed.

2. Each of the three parts of this medium was essential.

3. The essential nature of the yeast extract was because of its vitamin B_1 or vitamin thiazole content.

4. Unlimited growth of excised tomato roots appeared possible in a solution of mineral salts, cane sugar, and vitamin B_1 or thiazole.

5. The following compounds in the amounts used did not substitute for vitamin B_1 or the vitamin thiazole in the growth of excised tomato roots: indole(3)acetic acid, yeast ash, vitamin C, pantothenic acid, vitamin G (B_2), asparagine, cysteine hydrochloride, inositol, urea, pimelic acid, hydrolyzed nucleic acid.

6. Neo-peptone, malt flour, and certain samples of maltose at suitable concentrations substituted for yeast, probably because of their vitamin B_1 or thiazole content.

7. The growth of excised tomato roots was affected by extremely small amounts of vitamin B_1. The effect of 0.000000001 mg. (1 part in 40,000,000,000,000 parts of culture solution) was observed. It is believed, however, that the presence of 0.1 gamma or more is necessary for unlimited growth.

8. Tomato roots grown in a solution supplemented with thiazole alone synthesized the pyrimidine intermediate of vitamin B_1.

9. Vitamin B_1 did not replace inorganic nitrogen nor inorganic sulphur for tomato roots.

10. Excised tomato roots apparently required no other source of nitrogen than nitrates for unlimited growth.

11. Cane sugar, glucose, levulose, maltose, or cellobiose were available carbon sources for excised tomato roots.

12. The contaminants in "pure" sugars markedly affected the growth of excised tomato roots.

13. WHITE's mineral solution was not adequate for best growth of tomato roots.

14. The addition of boron and zinc improved WHITE's mineral solution.

15. Certain samples of filter paper and of filter paper ash improved WHITE's solution.

16. A modified Pfeffer's solution with 2.5 p.p.m. of $Fe_2(SO_4)_3$ instead of 0.8 p.p.m. $FeCl_3$ was superior to WHITE's mineral solution when yeast extract was replaced by vitamin B_1.

17. Excised tomato roots did not require light for unlimited growth.

18. Variability in the growth of individual roots under the same conditions may have been associated with differences in the composition of the fragments used as inoculum.

NEW YORK BOTANICAL GARDEN
NEW YORK CITY, N. Y.

DEPARTMENT OF BOTANY
UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI

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CROSSING-OVER, FRAGMENTATION, AND FORMATION OF NEW CHROMOSOMES IN AN ALLIUM SPECIES HYBRID[1]

S. L. EMSWELLER[2] AND H. A. JONES[3]

(WITH FOURTEEN FIGURES)

Introduction

The chromosomes of *Allium* species, because of their large size and conspicuous constrictions, are exceedingly favorable for cytological studies. As pointed out by LEVAN (**18**), they may be grouped under three general types: those with median insertions which divide them into arms less asymmetrical than 2:3; those with arms showing greater asymmetry than 2:3; and the subterminally constricted ones in which the shorter arm is less than one-fourth the length of the entire chromosome.

In an earlier paper (**9**) the writers discussed some cytological phases of a hybrid between *Allium cepa* L. and *A. fistulosum* L. The *A. cepa* parent was a strain of Yellow Globe Danvers (15–108–1), inbred two generations, and the *A. fistulosum*, a strain designated as 37–1. This paper is concerned almost entirely with the irregularities that occur in the same hybrid. Since *A. fistulosum* has been shown to be highly resistant to onion smut (*Urocystis cepulae* Frost), pink-root (*Phoma terrestris* Hansen), and thrips (*Thrips tabaci* Lind.), it was felt that a cytological study of the *cepa*×*fistulosum* hybrid should throw some light on the possibility of securing recombinations of the genes of the two species.

Recently LEVAN (**19**) has also published on the cytological behavior of a *cepa*×*fistulosum* hybrid, in which the commercial variety Braunschweiger was crossed with *A. fistulosum*. In some particulars the cytology of our hybrid closely parallels that of LEVAN'S, while in others there are considerable dissimilarities. Among these, the

[1] These investigations were started while the writers were at the University of California. They have been completed at the United States Horticultural Station, Beltsville, Md.

[2] Principal Horticulturist. [3] Principal Olericulturist.

most noteworthy are: the considerably higher frequency of first metaphase bivalents; almost complete absence of first metaphase polyvalents; high frequency of fragments and chromatids with two insertion regions, and greater fertility when used in backcrosses. These differences were noted by LEVAN and attributed by him to a closer relationship between our parental forms. That this is probably true is indicated by the even greater fertility of more recent hybrids made by us between other varieties of *cepa* and the same form of *fistulosum*. Likewise, additional hybrids of *fistulosum* with still other varieties of *cepa* have proved more sterile. Cytological observations of these have not yet been made, with the exception of a few slides from one between the *cepa* variety, Australian Brown, and *fistulosum*. MAEDA (**24**) has also recently published on the chiasmata of *cepa*, *fistulosum*, the F_1, F_2, and first backcrosses. He also reports the occurrence of fragments but makes no mention of chromatid bridges.

Methods

All the photomicrographs are from aceto-carmine smears made as described in an earlier paper (**9**). The post-meiotic chromosomes are from gentian violet smears prepared according to the method of LEVAN (**20**). The photomicrographs were taken with a Zeiss photomicrographic camera, on a Zeiss microscope with 15× ocular and 40× dry objective. The magnifications are *ca.* 600 and there has been no reduction in reproduction. The camera lucida drawings were made with a Zeiss microscope with 15× ocular and 90× 1.4 apochromatic oil immersion objective, giving a magnification of 2100 at table height. Reductions are given with each figure.

Investigation

MEIOSIS IN THE HYBRID

The somatic chromosome number in both *Allium cepa* and *A. fistulosum* is sixteen. The frequency of metaphase pairing in the hybrid was variable, as shown in table 3 of an earlier paper (**9**). There was a preponderance of complete pairing, however, the pollen mother cells with eight bivalents comprising slightly over 72 per cent of the 130 examined. In only one instance was a trivalent observed at first metaphase (fig. 3*A*). This situation differs strikingly

from LEVAN's hybrid, in which only 8 per cent of 100 cells studied contained eight bivalents. He also found in these cells three trivalents, two tetravalents, and one hexavalent.

The occurrence in our hybrid of chromatids with two insertion regions is fairly common in first anaphase and subsequent phases of meiosis. There is also a high frequency of fragments. The subsequent description of meiosis in the *cepa*×*fistulosum* hybrid is concerned mostly with the appearance and behavior of these irregularities and their bearing on formation of morphologically new types of chromosomes.

LEPTOTENE.—At leptotene it was impossible to observe any differences between the hybrid and parental forms. As reported in an earlier paper, the leptotene chromosomes appeared to be unsplit, but in the light of recent investigations by KOSHY (**16**), RUTTLE and NEBEL (**33**), and DERMEN (**8**), it may be that a different technique might have revealed a more complex structure.

ZYGOTENE.—At this phase of meiosis in the hybrid (fig. 1*A*) the first indications of irregularities were observed. It is very difficult to analyze them because of chromosome clumping. There are, however, many multivalent associations which give rise to considerable entanglement among the chromosomes. Frequently two chromosomes paired for a short distance separate, each then pairing with another. As a result unpaired areas such as shown at *a*, figure 1*A*, are rather common at this stage. This is in strong contrast to both *fistulosum* and *cepa*, where similarly unpaired chromosome sectors were not observed. LEVAN (**19**) reports the same situation in his hybrid.

PACHYTENE.—At pachytene, multivalent associations were also common. Occasionally a cell was observed that closely resembled the condition in "sticky chromosome" plants of *Zea mays* as described by BEADLE (**2**). A later stage (fig. 2*E*) closely resembles his anaphase group of plate 1, figure 30. It is possible that some of the apparent multivalent associations at the pachytene and earlier stages are not actual pairings but chromosome overlaps. This condition is undoubtedly accentuated by the flattening of some of the pollen mother cells; but even accepting this possibility, there are many unquestionable multivalent associations. The situation is

very similar to pachytene pairing in a diploid *Allium schoenoprasum*, following exposure of umbels to x-ray treatment (**22**). The *schoenoprasum* flowers were of various sizes, so that all phases of meiosis were subjected to treatment. Pollen mother cells were fixed daily for 4 days after irradiation. One plant which was exposed to Roentgen rays for only 15 minutes showed the following on a slide made on the third day following treatment: "Very clear pachytene stages of this specimen frequently showed examples of abnormal pairing of the threads. Trivalent and quadrivalent configurations occurred, as well as bivalents with inversions." These irregularities in *schoenoprasum* strongly suggest translocations which are known to occur rather frequently as a result of irradiation.

In the *cepa*×*fistulosum* hybrid described in this paper a few fragments were found at pachytene. They were completely separated from the remainder of the chromosomes, and because of their small size could not have been univalents or foldbacks. While univalents were also common, they could usually be identified because of their larger size. Classification as a fragment is necessarily very broad, since any piece of a chromosome regardless of size may be considered one. In this paper only those clearly smaller than any of the parental chromosomes are classed as fragments. They may or may not have an insertion region. Undoubtedly the actual number, when interpreted in the broad sense, was much higher.

DIPLOTENE.—At diplotene multivalents are found less frequently, indicating that dissociation of earlier pairing has taken place. In many instances the chromosomes form a tangled mass (fig. 1*B–F*). Unfortunately the insertion regions of *Allium* chromosomes are not recognizable in early meiotic stages. Because of this it cannot be determined whether they are always exactly opposite each other in paired chromosomes. The size and morphology of the chromosomes of the two species are so similar, however, that such conditions as depicted at *a*, figure 1*D*, where in two bivalents the end of one chromosome extends beyond the other, indicate that insertion regions of paired chromosomes may sometimes not be opposite each other. Instances of this sort are common in this hybrid. In some cases a chromosome extends beyond the one with which it is synapsed and pairs with a third. This is true of the lower of the two in *a*, figure

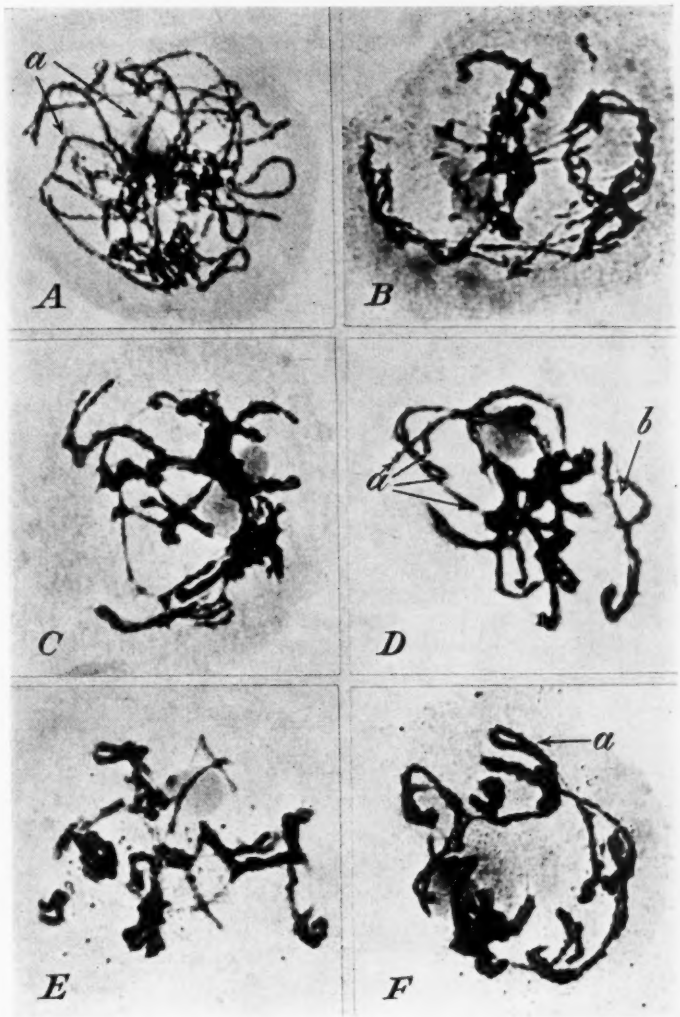

FIG. 1.—Multivalent associations at zygotene and diplotene: *A*, unpaired threads at *a* in zygotene; *B*, *C*, *D*, *E*, diplotene showing entanglement of chromosomes forming multivalent associations.

1*D*. Another configuration seen occasionally is that shown at *b*, figure 1*D*. The loop may be the result of an inversion. At *a*, figure 1*F*, the loop suggests a duplication. In figure 2*B*, *C*, and *D*, multivalent associations are very clear. None of these irregularities were observed in either *cepa* or *fistulosum*.

The occurrence of fragments (*f*) at diplotene is shown in figure 2*A–D*. They are undoubtedly fragments, size alone precluding any possibility of their being univalents. On the same basis, the possibility of their being foldbacks can also be eliminated. Lest they may have been caused by mechanical injury as a result of the method of making slides, a great number of observations were made on similarly prepared material of the parent forms of *Allium cepa* and *A. fistulosum*. No fragments were found in *A. fistulosum* and but one in *A. cepa*. In the latter instance it occurred at late first anaphase and was associated with a chromosome bridge. These observations indicate that fragments are typical for this hybrid.

DIAKINESIS.—By the time diakinesis is reached, the chromosomes are well spaced and can readily be determined as univalents or bivalents. The time required for this phase of meiosis is probably comparatively short, since the number of diakinesis figures observed was considerably smaller than any other stage. Polyvalent associations are rare, only one being observed in seventy-five cells. It is shown in *a*, figure 2*F*. Here each end of a chromosome is paired with two others that lie some distance from each other. As a result the connecting chromosome is pulled out across the cell. The blurred spot at the right was caused by extraneous material on the under side of the cover slip. The loop at *b* is a type of configuration that occurs rather frequently. It is essentially the same configuration as shown at *b*, figure 1*D*.

FIRST METAPHASE.—At metaphase the chromosomes in 232 cells, with the exception of the one trivalent shown at *a*, figure 3*A*, were either univalents or bivalents. The bivalents form one of two general types of configurations, either rods or rings (fig. 3*B*). All the chiasmata become terminal as in *A. cepa*, and the hybrid metaphase plates, in which irregularities do not occur, are almost identical with those of *A. cepa*.

The bivalents of the hybrid and *A. cepa* differ markedly from

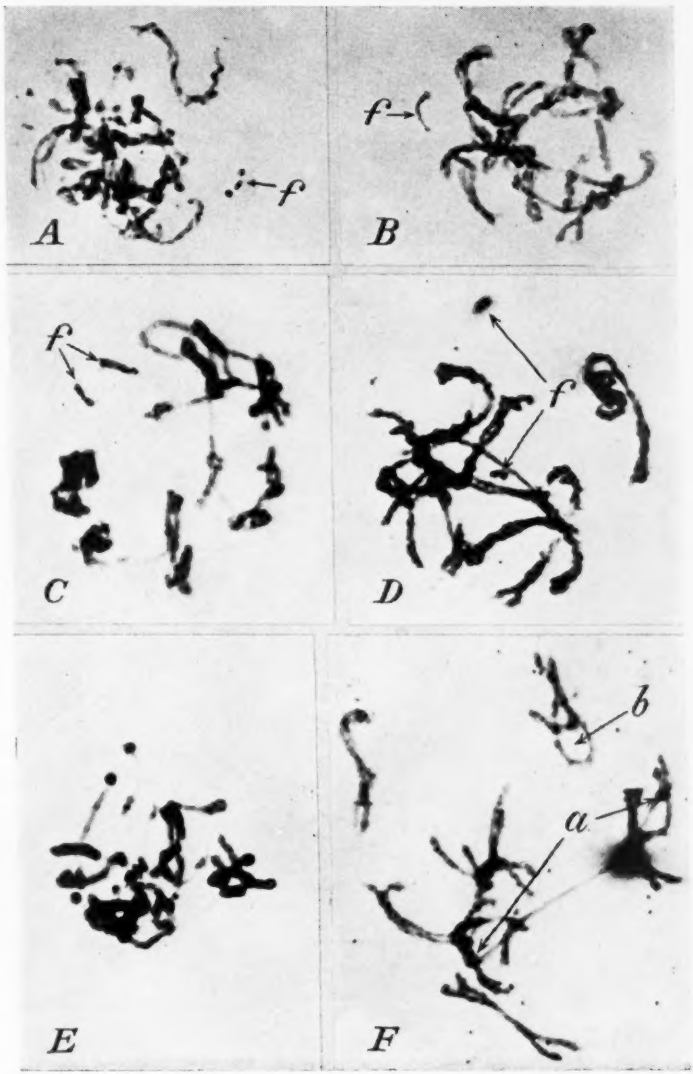

FIG. 2.—*A*, *B*, *C*, *D*, showing fragments (*f*); *E*, entanglement very similar to "sticky gene" situation in *Zea mays; F*, multivalent association at diakinesis.

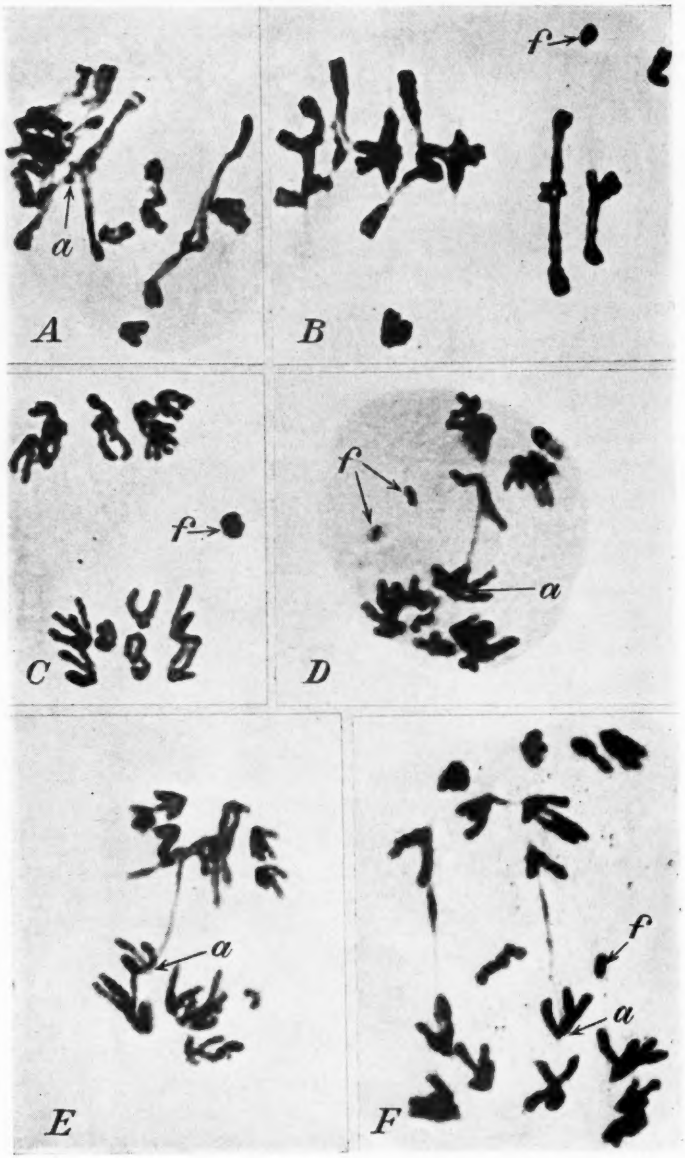

FIG. 3.—*A*, trivalent at first metaphase; *B*, fragment and univalents at first metaphase; *C*, fragment at first anaphase; *D*, *E*, *F*, bridges at first anaphase. Note three free arms at each pole.

those of *A. fistulosum*. As shown by ISHIKAWA (**14**), LEVAN (**21**), and EMSWELLER and JONES (**9**), this variation between the species is caused by differences in location of chiasmata at first metaphase. In *A. cepa* at early metaphase they are randomized, while in *A. fistulosum* they are localized at the insertion region. In *cepa* they later become terminal, in *fistulosum* they do not. EMSWELLER and JONES (**10**) recently presented data which indicated that localization in *A. fistulosum* was probably controlled by a recessive gene. LEVAN (**18**) has found a somewhat similar situation in *A. nutans*, there having appeared a plant with localized chiasmata in an inbred line of this species which normally shows only a random arrangement of the chiasmata at first metaphase.

At first metaphase there are many more cells containing fragments than at diplotene and diakinesis. A metaphase group with a fragment (*f*) is shown in figure 3*B*. There are seven bivalents, two univalents, and one fragment. Fragments of this type are relatively easy to determine. It is only when they are almost as large as a univalent that difficulties arise.

FIRST ANAPHASE.—The first anaphase figures may be grouped into two classes, those with chromosome bridges and those without. In figure 3*C* there are eight chromosomes at each pole and a fragment lying near the equatorial plate. This type of first anaphase was not uncommon; that is, there were many cells without bridges but containing one or more fragments.

First anaphase groups with one bridge are shown in figure 3*D* and *E*. Two bridges occur in *F*. In *D* and *F* there are unmistakable fragments, and in *E* to the right of the lower group of chromosomes there is a small mass of chromatin, which appears to be a univalent but may be a large fragment. At *a*, in each of these three figures, it can be seen that the bridge is simply a chromatid with two insertions, which are moving to opposite poles. At each end of the bridge a chromatid with one attachment is clearly seen held fast to the doubly constricted one at the insertion region. Close examination of these figures shows three free chromosome arms at each pole with the fourth stretched out to form a so-called bridge. Such a bridge then consists of three chromatids, two having one insertion each, while the third has two.

FIRST TELOPHASE.—A "normal" first telophase group is shown in figure 4*A*. There are no visible fragments in this cell, but in many similar cells they do occur, sometimes masked at one or both poles by the darkly staining group of chromosomes. In figure 4*B* two small fragments (*f*) can be seen at the left, and either a larger one or a univalent at the right (*a*). It is clear that separation of the latter is being completed.

The first telophase groups of figure 4*C* and *D* contain a chromosome bridge connecting the chromatin masses at each pole. In *E* there are two bridges and in *F* there are three. In *C* no fragments are found. It is possible, however, that one might be included in the deeply stained chromatin at either pole, or have disintegrated before this stage of division was reached. In *D* a small fragment occurs near the bridge. The two bridges in *E* are accompanied by at least one fragment, and there is another small one and possibly a dividing univalent in *F*.

Occasionally there occurs a cell containing a great number of fragments. One is shown in figure 5*A*. There are seven unquestionable pieces of chromosomes, and two others may be univalents but are probably large fragments. What appears to be the remnant of one bridge is seen at *a*.

FIRST INTERPHASE.—At first interphase, when fragments occur, the cell may or may not contain one or more bridges. In figure 5*B* there are five visible fragments and what may be the remnant of a bridge (*a*) jutting out from the lower nucleus. In *C* there is one fragment and probably a univalent. The bridge in *D* was not accompanied by a fragment, whereas in *E* a small one is present.

The behavior of chromosome bridges during the first interkinesis was given considerable study. In figure 5*D* and *E* the outlines of intact ones can readily be seen. The chromatid with two insertion regions has persisted as a connection between the daughter interkinetic nuclei. Within each nucleus the identity of the chromosomes is almost obliterated. In slightly earlier stages (fig. 4*C–F*) the bridges are intact and stain as deeply as the telophase chromosomes at each pole. In other first telophase cells (figs. 5*F*, 6*A*, *B*, *C*), however, the bridges do not stain so deeply and do not extend completely across the cell as a connection between the newly formed daughter

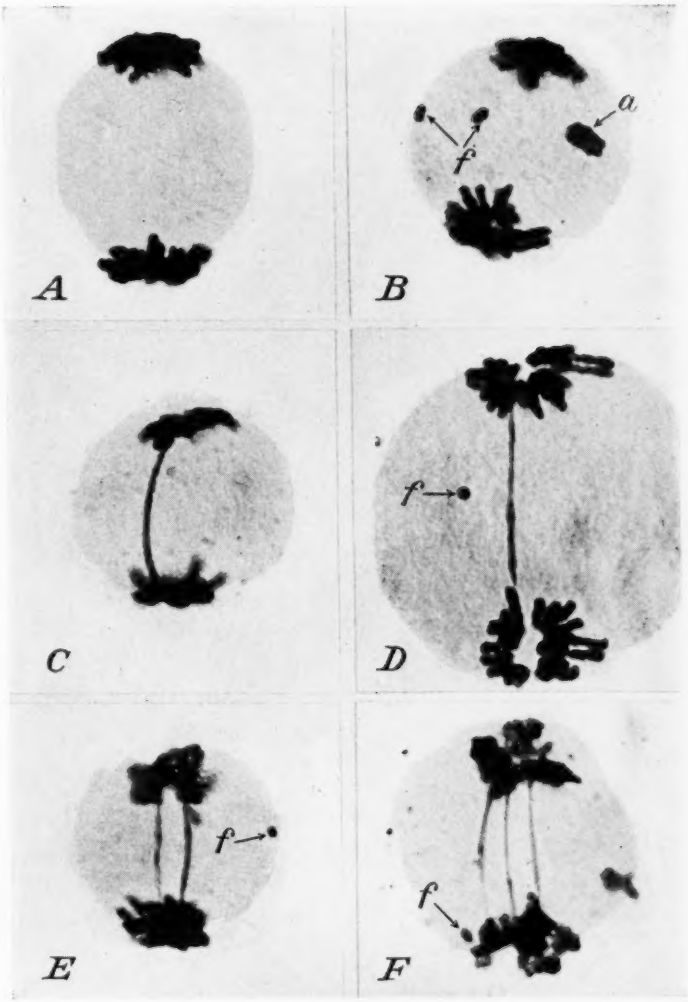

FIG. 4.—First telophase: *A*, normal figure without bridges or fragments; *B*, two fragments and univalent; *C*, chromatid bridge but no visible fragment; *D*, chromatid bridge and fragment; *E*, two chromatid bridges and one visible fragment; *F*, three chromatid bridges and one visible fragment.

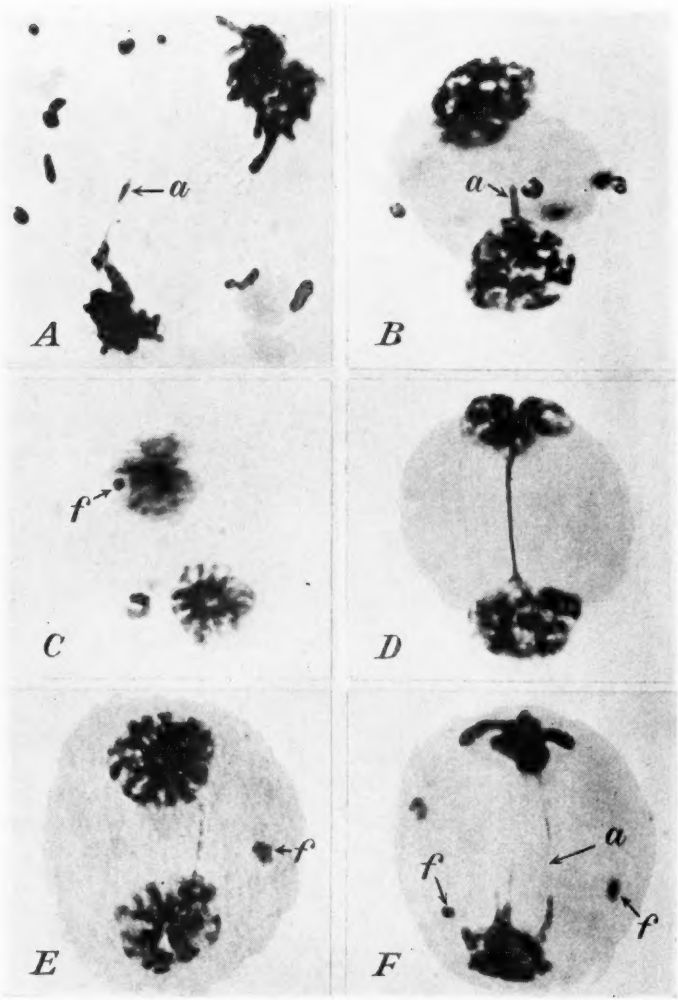

FIG. 5.—*A*, late first telophase showing numerous fragments and remnant of chromatid bridge; *B*, first interphase showing five visible fragments and remnant of bridge; *C*, fragment and univalent but no bridge; *D*, chromatid bridge but no visible fragment; *E*, disintegrating bridge and fragment; *F*, remnants of three bridges showing disintegration of central portions.

nuclei. In 6*B* and *D* a piece of a chromatid can be seen midway between the new daughter nuclei. The remnant of a bridge projects from each nucleus in *D*, and undoubtedly the piece of chromatid (*a*) was originally a part of this bridge. The cytological evidence indi-

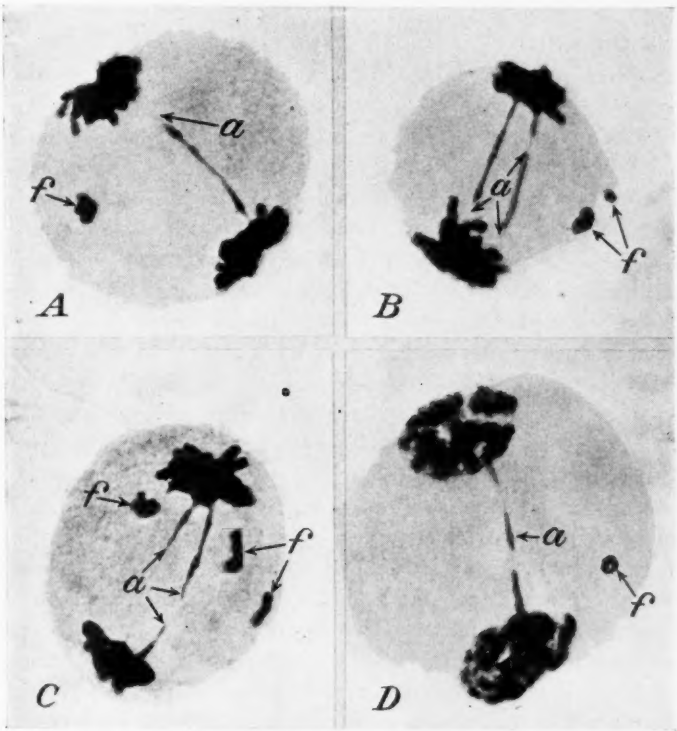

FIG. 6.—*A*, chromatid bridge disintegrating near upper nucleus; *B*, two bridges, one freed at one end, the other at both ends; *C*, two bridges, one almost completely lost, second with small portion lost near lower nucleus; *D*, first interphase with bridge "broken" at two points, leaving piece out in center.

cates that a bridge may become disconnected at one or more points between the daughter nuclei. There is no indication that cytokinesis has been initiated as yet, therefore it cannot be responsible for the breaks in the bridges.

SECOND METAPHASE.—A second metaphase is shown in figure 7*B*. The pollen mother cell wall has not been ruptured and the daughter cells are not flattened out. Chromosomes with two insertion regions were not observed with certainty in second metaphase groups, but wider search should have discovered some, since the occurrence of bridges at second anaphase indicates that they sometimes reach one or the other pole.

SECOND ANAPHASE.—At second anaphase the bridge configurations are distinctly different from those of the first division. They are formed by one chromatid which has two insertion regions, and the two sister chromatids with but one are no longer attached to it, having been released by separation of the insertion regions, thus completing the mitotic split. This condition is shown at *a* in figure 7*C*. The sister anaphase cell did not have a bridge, so the one shown in the photomicrograph was freed from it in order to be more easily flattened for photographing. In the cell shown there are seven chromosomes at each pole. The bridge is composed of a chromosome with two insertion regions, which were so oriented at this division that they went to opposite poles. A comparison of this figure with figure 3*D*, *E*, and *F* will demonstrate the differences described.

In figure 7*A*, cytokinesis has occurred and the second anaphases are actually in separate cells, although they were both held within the pollen mother cell wall, which was ruptured, allowing the chromosomes to be flattened out. Two fragments are included within one of the new cells and three within the second. Some of the configurations at the left are certainly suggestive of chromatid bridges.

A very interesting second anaphase group is shown in figure 7*D*. There are seven fragments and seven bridges, three of which are interlocked. Cytokinesis has not occurred and the interlocked chromatids with two insertion regions have persisted into the second division. The probability of this anomaly is certainly very low, since it represents the formation of seven chromatids with two insertion regions out of a total of eight possibilities. There must also have occurred an interlock of two first anaphase chromatids, each of which had acquired two insertion regions. The situation is shown more clearly in the diagrammatic drawing of figure 8. There must

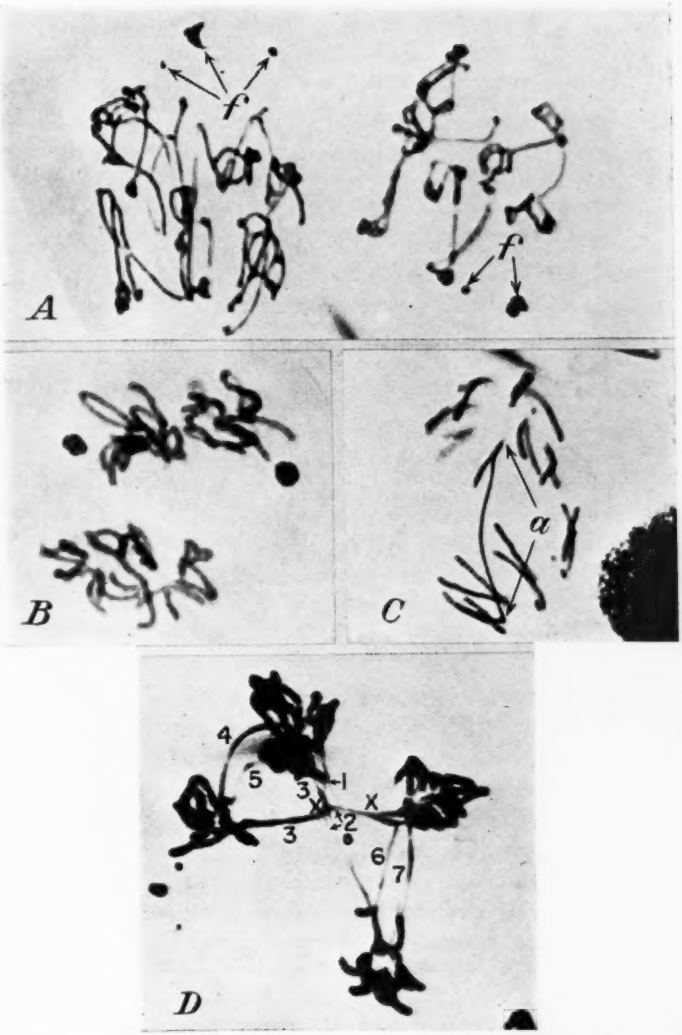

FIG. 7.—*A*, second metaphase showing fragments (squeezed out of p. m. c. wall); *B*, same stage in p. m. c. wall; *C*, second anaphase showing structure of chromatid bridge at this stage of meiosis (note that it is simply a single chromatid); *D*, complex second anaphase group showing interlocking of chromatid bridges (see fig. 8).

have been at least seven chromatids with two insertions at first metaphase, and there would have been but one bridge at first anaphase had interlocking not occurred. In the diagram of first telophase (fig. 8) the only true bridge is numbered 1. It is interlocked with chromatid 2 at X, and chromatid 2 is also interlocked with 3 at X'. The two insertions of 2 were oriented to one pole and those of 3 to the other at first anaphase. These chromatids would not have appeared at this division if the interlock at X' had not occurred. There might have been some difficulty at X, but it is likely that chromatid 2 would have slipped along 1 and been included in the

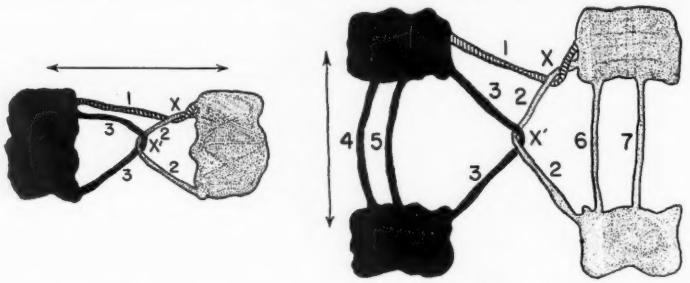

FIG. 8.—Diagrammatic sketch of D in fig. 7: first anaphase at left and second at right. Arrows denote planes of divisions.

new daughter nucleus. The other four bridges, 4, 5, 6, and 7, did not appear until the second anaphase, which was oriented as shown by the arrow. Cytokinesis did not occur following the first division, otherwise the interlocked chromatids would not have persisted. Possibly such an unusual condition prevented a normal first interkinesis and hastened the second division.

SECOND TELOPHASE.—A second telophase without bridges is shown in figure 9A. There is at least one and perhaps two fragments. The same stage with a bridge in each anaphase group is shown in 9B. Fragments were not visible in this figure, but might have been included in any of the four masses of chromatin or have disintegrated by this time. Two fragments in C are excluded from the telophase groups, and two bridges are visible at the right.

The behavior of bridges at the second telophase is essentially the same as at the first division. Some persist for a long time and show all the conditions as described earlier for the first division.

SECOND INTERPHASE.—A second interphase is shown in figure 9*D*. At least three fragments are visible. In this instance all three are outside the nuclei and may form micro-nuclei. At this stage also the remnants of bridges may sometimes be seen jutting out from

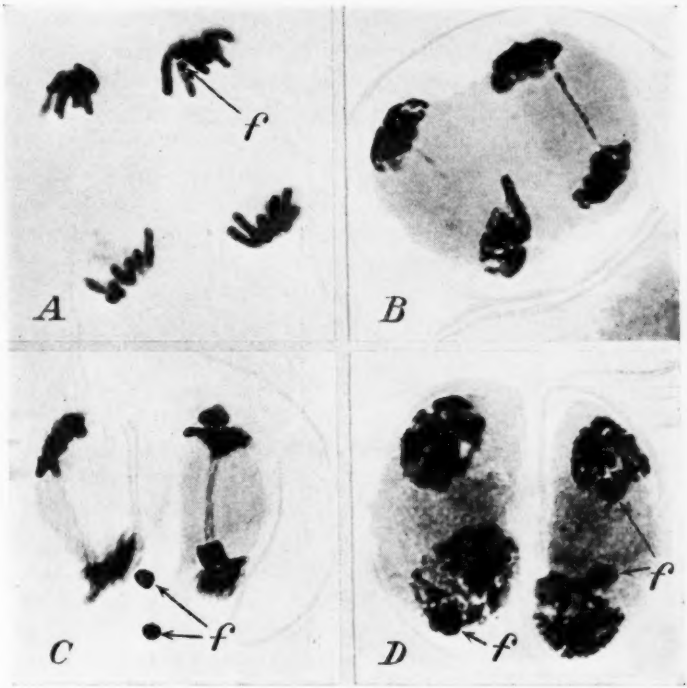

FIG. 9.—*A*, second anaphase showing one visible fragment and no bridges; *B*, bridge in each division but no visible fragments; *C*, one bridge at left and two at right, also two fragments; *D*, second interphase with three visible fragments and no bridges.

daughter nuclei. Intact bridges were not observed at second interphase. In many instances fragments become included in the second interphase nuclei and persist into the microspores.

POST-MEIOTIC CHROMOSOMES

The first post-meiotic mitosis in *Allium cepa* and *A. fistulosum* can be studied with comparative ease. The division takes place soon

after the four microspores are released from the microsporocyte. When the material is favorable it is possible to find many excellent metaphases on one slide. It was more difficult, however, to find good figures in the hybrid.

A count of the chromosomes in eighty-seven dividing microspores is summarized in table 1. There were seven different classes, the lowest number of chromosomes being six and the highest sixteen. Those with eight comprised the largest class, while those with nine

TABLE 1

CHROMOSOME NUMBER OF MICROSPORES, AND BIVALENT FORMATION IN THE HYBRID

No. of chromosomes in microspore	Frequency	Percentage	Microspores with fragments	Percentage	Type of pairing	Frequency	Percentage
6	2	2.28	0	.....	.....	.....	.....
7	1	1.14	1	.....	.....	.....	.....
8	59	67.81	10	16.94	$8_{II}-0_I$	94	72.30
9	15	17.24	4	26.66	$7_{II}-2_I$	24	18.46
10	6	6.89	2	33.33	$6_{II}-4_I$	9	6.15
11	1	1.14	1	.....	$5_{II}-6_I$	1	0.76
.....	.....	.....	.....	.....	$4_{II}-8_I$	2	1.53
16	3	3.42	0	.....	.....	.....	.....
Totals	87	.....	18	.....	.....	130	.....

were next numerous. There were three giant pollen grains with sixteen chromosomes which were spherical in shape, whereas the haploid and aneuploid types had the appearance of a slightly flattened ellipse. No observations were made on the mode of origin of these large pollen grains. It is interesting to note that microspores with less than the haploid number are able to survive thus far and to appear normal in every respect except chromosome number. It is not known whether such microspores can function, but the evidence on this point is meager. As noted earlier (9), the hybrid is almost completely egg sterile, but slightly pollen fertile. The backcrosses to *A. fistulosum* and *A. cepa* so far studied have all contained sixteen chromosomes. The number of backcross plants examined has not been large, however, and the data, although highly suggestive,

cannot be accepted as conclusive evidence that heteroploid microspores are not viable.

Included in table 1 are data on frequency of bivalent formation in the hybrid. The three most frequently occurring numbers are: eight bivalents, seven bivalents plus two univalents, and six bivalents plus four univalents. This is interesting when compared with the situation in the microspores. Here the three largest classes are eight, nine, and ten chromosomes. The three largest groups, both as to bivalent formation and chromosome number, are also expressed in percentage and there is close agreement between these figures. The microspores with eight chromosomes may arise following any type of pairing, and a higher percentage than actually found would not be unusual. There should also be approximately as many microspores with six and seven chromosomes as with ten and nine. Their absence is to be expected, however, since in general, reduction of chromatin in gametes and zygotes has greater lethality than addition.

In comparing the morphology of the chromosomes of *Allium cepa* with those of *A. fistulosum*, EMSWELLER and JONES (9) used the ratio of the length of the short to the long arm to secure an index number for each chromosome.

Twelve dividing microspores in the hybrid were selected for a critical study of the chromosomes they contained. They were chosen primarily because nearly all the chromosomes were flattened out, which is absolutely essential if arm measurements are to have much value. Since the frequency of bivalent formation in the hybrid was so high, it is reasonable to suspect that considerable crossing-over has taken place. As a result there have been formed several new chromosome types, which are shown in figures 10 and 11. In every instance the length of each arm was determined by measuring metaphase chromosomes of the first microspore division projected upon a sheet of paper with a Zeiss microprojector. The measurements of the chromosomes of *A. cepa* and *A. fistulosum* represent the average from eleven and nine cells respectively in which all the chromosomes were well flattened. The measurements from the hybrid represent single microspores, of course, and in some instances all the chromosomes of a cell were not ideally spaced or flattened. Each chromo-

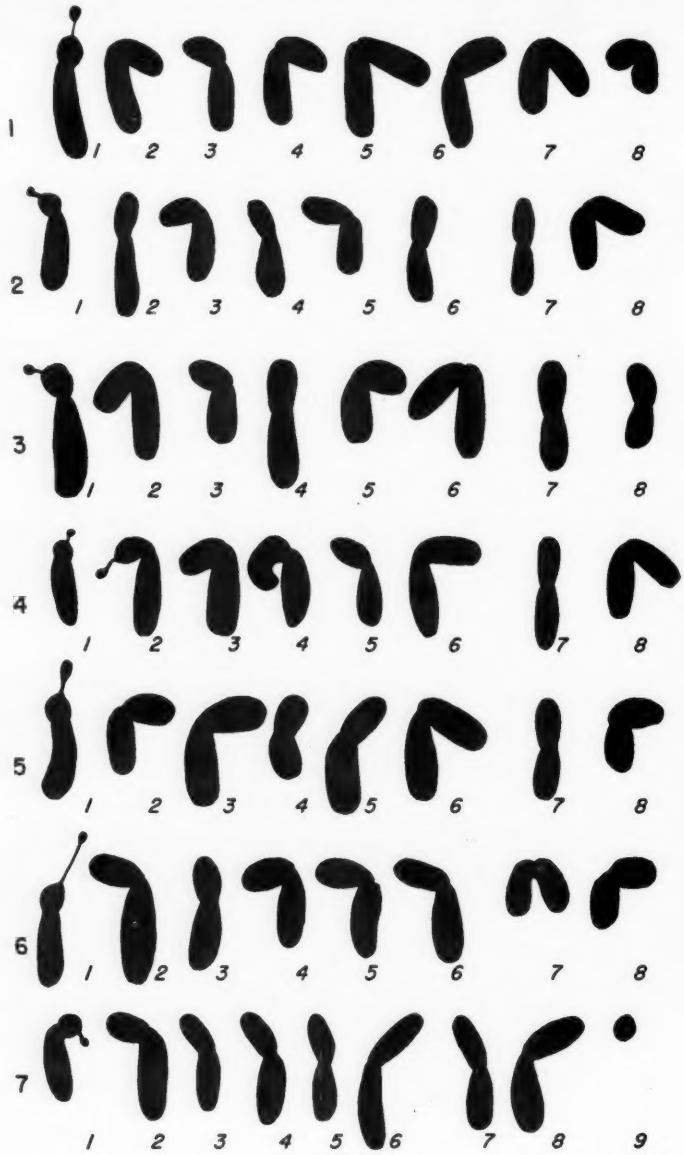

FIG. 10

FIGS. 10, 11.—Post-meiotic chromosomes in *Allium cepa*, *A. fistulosum*, and their hybrid.

8
1 2 3 4 5 6 7 8
9
1 2 3 4 5 6 7 8 9
10
1 2 3 4 5 6 7 8 9
11
1 2 3 4 5 6 7 8 9 10
12
1 2 3 4 5 6 7 8 9
13
1 2 3 4 5 6 7 8 9 10 11 12 13
15
1 2 3 4 5 6 7 8 9 10

FIG. 11

some, however, was measured at least fifteen times and the mean computed. The ratio of the short to the long arm was calculated and the data are recorded in table 2. In figures 10 and 11 the chromosomes are arranged and numbered in the order in which they appear in table 2. The one with the most nearly terminal constriction is at the left and the most nearly median at the right. The arrangement is not intended to signify homologies.

The morphology of the chromosomes in the microspores of the hybrid indicates that crossing-over was rather frequent. Unless both are changed proportionately, any modification in length of chromosome arms of course will result in a change in the ratio of the short to the long. Table 2 in conjunction with figures 10 and 11 indicates that such modifications have occurred. As shown in an earlier paper (9), the chromosomes of *A. cepa* and *A. fistulosum* may be placed in three groups, according to the location of the insertion region. There are in each species four median chromosomes, three submedian, and one subterminal, which has a satellite on the proximal arm.

In the description of each hybrid microspore, only the more easily distinguished new chromosomes are discussed. It is very probable that chromosomes other than those noted here were also modified in shape, but the changes are so slight that they could not easily be identified.

Microspores 1 and 2 are *cepa* and *fistulosum* respectively. In microspore 3, which is from the hybrid, there are five median, two submedian, and a satellited subterminal chromosome. Included are three large chromosomes, 1, 4, and 6, which are probably new types. The satellited chromosome resembles that of *cepa*, but the length of the distal in proportion to the proximal arm is greater and the index number accordingly reduced. Chromosome 4 is about the same length as *cepa* 5 and *fistulosum* 2, but its index number places it between them as to relative length of arms. Number 6 has its insertion more median than *cepa* 5 or 6, and is probably too long for *cepa* 7 or 8.

In microspore 4 there are two satellited chromosomes. It is the only instance in which two such chromosomes occurred in the same genom. Very probably this microspore arose from a pollen mother

cell in which not more than seven bivalents were formed, with the satellited chromosomes remaining unpaired and migrating to the same pole. It appears from table 2 that the satellited chromosomes in the hybrid microspore are shorter than in the parent species. Since even the same chromosome is known to vary in length in various cells of the same plant, these differences are probably of no significance. The index numbers indicate that chromosome 1 of the hybrid microspore is the *fistulosum* satellited chromosome and that number 2 is the unmodified *cepa* one. The deviation of number 1 from *fistulosum* (0.252–0.248) is within the range secured when nine *fistulosum* satellited chromosomes in as many microspores were measured. The actual figure for chromosome 1 of *cepa* was 0.3661, and for the second satellited chromosome of microspore 4 it was 0.3665. This is also well within the range secured from index numbers of eleven *cepa* satellited chromosomes. In addition to the evidence of index numbers, a difference in diameter of the two chromosomes also exists comparable with that between the parental chromosomes. This condition was noted by LEVAN (**19**). In his hybrid, the *fistulosum* chromosomes had a smaller diameter than *cepa*. Chromosome 1 is not so broad as 2, which fact further identifies it as the *fistulosum* satellited chromosome.

In genom 5 there are several deviations from parental chromosome types. The satellite is elongated and larger than that of either parent, and all the other chromosomes have median or nearly median insertions. The index numbers of 2, 3, 4, 5, and 6 are very similar and it would be difficult to distinguish between 2 and 4 and between 3, 5, and 6. Both 7 and 8 have fully median insertions, and certainly one is a new type since there was only one perfectly median chromosome in the hybrid. This was number 8 of *fistulosum*.

The satellited chromosome of microspore 6 has an unusually long attachment thread. The second one is much larger than any measured in *cepa* or *fistulosum*. Numbers 3, 4, 5, and 6 have very similar index numbers, and it would be difficult to distinguish among 3, 5, and 6 since they all have the same length. Some are probably new types.

In microspore 7 there is a small fragment. It has the spherical shape common to nearly all the fragments found in the microspores.

TABLE 2

LENGTH AND RATIO OF SHORT TO LONG ARM IN CHROMOSOMES OF ALLIUM CEPA, A. FISTULOSUM, AND POST-MEIOTIC CHROMOSOMES OF THEIR HYBRID

Chromosome no.	Cepa		Fistulosum		Hybrid genoms									
	1		2		3		4		5		6		7	
	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.
1	38.8	0.366	30.2	0.248	41.9	0.309	27.3	0.252	33.6	0.287	33.0	0.353	27.0	0.350
2	29.5	0.546	39.5	0.490	37.8	0.587	34.3	0.366	29.0	0.705	47.0	0.516	37.0	0.480
3	31.7	0.547	29.4	0.598	25.8	0.632	31.9	0.450	41.0	0.708	37.0	c.658	31.5	0.657
4	31.5	0.599	31.7	0.642	41.3	0.652	31.3	0.526	23.5	0.740	33.0	0.650	37.0	0.761
5	42.4	0.752	36.8	0.769	27.7	0.688	29.6	0.681	38.0	0.767	37.0	0.660	34.0	0.789
6	38.6	0.754	29.5	0.788	43.0	0.791	42.4	0.758	43.0	0.791	37.0	0.681	47.5	0.862
7	28.5	0.786	32.5	0.868	34.2	0.932	37.0	0.850	33.0	1.000	28.0	0.806	36.5	0.871
8	18.4	0.851	31.6	1.000	26.0	0.940	35.8	0.854	25.0	1.000	27.0	0.928	44.0	0.913
9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
Total lengths..	261.2	.....	259.4	.....	277.7	.....	269.6	.....	266.1	.....	279.0	.....	294.5	.....

TABLE 2—*Continued*

Chromosome no.	Hybrid genoms													
	8		9		10		11		12		13		15	
	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.	Length (mm.)	Index no.
1	40.5	0.500	33.0	0.375	49.0	0.400	32.5	0.585	34.0	0.545	61.5	0.366	36.5	0.303
2	38.5	0.604	18.0	0.384	24.5	0.441	26.0	0.588	34.5	0.682	50.5	0.443	47.5	0.461
3	35.0	0.666	51.5	0.745	21.5	0.653	27.0	0.625	17.0	0.700	33.0	0.609	35.5	0.510
4	34.0	0.837	30.5	0.794	35.0	0.666	28.5	0.628	26.0	0.733	31.5	0.615	48.5	0.616
5	40.0	0.860	33.5	0.914	28.5	0.676	31.0	0.631	27.5	0.833	34.0	0.789	37.5	0.875
6	32.0	0.882	25.0	0.923	19.5	0.772	44.0	0.739	35.0	0.842	20.0	0.818	47.0	0.880
7	23.0	0.916	43.5	0.934	31.5	0.800	25.0	0.785	21.5	0.869	32.5	0.857	28.0	1.000
8	.....	.....	33.0	0.941	33.0	0.833	28.0	0.793	35.5	0.918	40.0	0.904	28.0	1.000
9	.....	.....	.....	.....	30.0	1.000	40.0	1.000	.....	.....	23.0	0.916	28.0	1.000
10	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	21.5	0.954	.....	1.000
11	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	40.0	1.000	.....	.....
Total lengths	243.0	.....	268.0	.....	272.5	.....	276.0	.....	231.0	.....	377.5	.....	336.5	.....

Chromosomes 6, 7, and 8 all have a nearly median insertion. They are larger than the three corresponding types of *cepa* and *fistulosum* and are undoubtedly new types. There is also considerable variability in breadth among the chromosomes of this genom. Numbers 1, 2, 4, and 8 are all broader than the remaining four, 6 especially being noticeably more slender.

The situation in number 8 is interesting. All the chromosomes are shorter and thicker than in any other microspore. The absence of a satellite cannot be accepted with certainty, since it might have escaped observation in some way. It is probable, however, that a satellited chromosome was not included in the nucleus which gave rise to this group. This is the more likely explanation since there are but seven chromosomes and one fragment, and none of the seven has an index number approaching that of the satellited chromosomes of the parent species. The seventh chromosome is rather unusual; the knob on one arm is too large for a satellite and it appears to be a definite part of the main chromosome body, although it is somewhat smaller in breadth than the remainder. The same general form is also found in microspore 12. It is possible that these chromosomes may be types with secondary constrictions.

In microspore 9 the chromosomes vary considerably in size and index numbers. Numbers 5 and 8 are very similar in both length and arm ratios, but all others are easily distinguished. Number 2 is about the same size as 8 of *cepa* but is decidedly subterminal rather than median. The breadth of the proximal knob is also less than that of the distal arm. In this respect it is similar to chromosome 7 of microspore 8, and 3 of microspore 12. Number 3 is the second largest new chromosome observed in the hybrid microspores. It is exceeded only by 1 of genom 13. There is also a fragment present in this group.

There are nine rather distinct chromosomes in microspore 10. Number 1 is much larger than all the others and is subterminally constricted. It approaches the regular *Allium* satellite type, distinguished as the s chromosome by LEVAN (**18**). Chromosome 2 bears a satellite and has the largest index number of any satellited chromosome. In this group only 7 and 8 are difficult to distinguish.

They are nearly the same length and have somewhat similar index numbers. Number 9 may be an unmodified chromosome 8 of *A. fistulosum*.

The chromosomes of genom 11 do not contain a satellite type. Number 1 has an arm ratio of 0.585 while that of the *A. cepa* and *A. fistulosum* satellite-bearing chromosome is 0.366 and 0.248. The index number for chromosome 1 is in fact larger than the first *A. cepa* and the first and second *A. fistulosum* chromosomes. Chromosomes 2, 3, 4, and 5 would be somewhat difficult to distinguish. Number 9 with a fully median constriction is undoubtedly a new type. A fragment is present also in addition to the nine chromosomes.

The situation in microspore 12 is somewhat similar to that of 11. A satellite type chromosome is also missing. There is also more variability within the group as to length and breadth, and one less chromosome. Chromosome 3, which was mentioned earlier, is certainly a strikingly new type.

The largest new chromosome observed (number 1) occurs in microspore 13. It is about one-third larger than the longest in *A. cepa* and *A. fistulosum*, and is very much broader than any other chromosome. It is also a satellite type, having the same arm ratio as the *A. cepa* satellited chromosome. Chromosome 2 is the third largest and is also unquestionably a new type. The most unusual new chromosome observed is number 6 in this group. Each end bears a distinct satellite which differs in size in about the same degree as the satellites of *A. cepa* and *A. fistulosum*. A possible origin of this type will be discussed later in this paper. In addition to the eleven chromosomes there are two fragments.

In microspore 15, chromosome 1 is an unusual type. The main part is a satellite type chromosome and the portion attached to the proximal arm is very similar to chromosome 2 of genom 9. In this group there are also four medianly constricted chromosomes, 7, 8, 9, and 10. The first three have the same total length, but 7 is broader than the others. Number 10 is smaller, but would be difficult to distinguish from 7, 8, or 9. Since there was only one chromosome in the hybrid (number 8 of *fistulosum*) that had arms of equal length, at least three of these are new.

FRAGMENTATION AND CHROMOSOME BRIDGES IN THE HYBRID

The frequency of fragments at each phase of meiosis and in the microspores is shown in table 3. They appear first at late pachytene and from then on to the first mitotic metaphase of the microspore. Only two were found at pachytene, one each in two cells out of 223 observed. At diplotene the cells with fragments increased sharply to 31 in 449. This percentage is not changed at diakinesis, but increases greatly at first metaphase. The frequency is again increased

TABLE 3

FREQUENCY OF FRAGMENTS IN A. CEPA×FISTULOSUM HYBRID

STAGE IN MEIOSIS	CELLS WITH FRAGMENTS	CELLS WITHOUT FRAGMENTS	PERCENTAGE CELLS WITH FRAGMENTS
Leptotene	0	101	0.00
Zygotene	0	220	0.00
Pachytene	2	221	0.89
Diplotene	31	418	6.90
Diakinesis	7	99	6.60
First metaphase	128	521	24.56
First telophase	52	102	33.76
Second metaphase	61	200	23.37
Second telophase	43	134	24.29
First post-meiotic mitosis	18	69	20.68

at first telophase, but drops again at second metaphase, and at second telophase remains about the same. In the microspores there is a further drop.

There appear to be several points at which fragments are released, the first being between pachytene and diplotene. Apparently there is no further increase until following diakinesis, but at first metaphase the frequency is quadrupled. It is further increased between this point and first telophase, at which time more cells contain fragments than at any other phase of meiosis. In the second metaphase the frequency drops again and there is no significant difference on through second telophase. The frequency at the first microspore metaphase is somewhat misleading. Unfortunately the total number of fragments in the earlier stages was not recorded at the time the data were collected. Some of these had from three to five. When the four

microspores were released, some included one or more, either by accident or because of having received them at an earlier division. It must also be remembered that these counts are from static conditions at certain phases of meiosis. The fragments are actually being released at all times following late pachytene.

The fragments found at pachytene, diplotene, and diakinesis are probably the result of the many multivalents observed during early meiosis. They also probably lack an insertion region and most likely have been formed as a result of crossing-over in possibly both homologous and non-homologous associations of terminal segments. The probable method is shown in figure 12, where two alternatives are diagrammed. In *A* and *B*, the metaphase association is shown at *m*, and the anaphase chromosomes at a_1 and a_2. In *A*, chromosome *a*–*a* forms a chiasma with *b*–*b* at *x*. If, following either a chromatid break or formation of a new one, the realignment and orientation are as shown, there results a long chromatid, a'–b', with two insertion regions, two unmodified ones, *a*–*a* and *b*–*b*, and a small fragment b'–a'. If, however, the realignment is as shown in *B*, there would be no chromatid bridge, but there would result, as shown in an earlier paper (9), a long+short chromatid to each pole and a small fragment. Fragments would probably be released shortly after being formed and probably account for most of those observed in this hybrid before first metaphase. Either method will account for the formation of chromosome 6 in microspore number 13.

For some reason as yet unknown, the *A* type does not seem to be common in this hybrid. Terminal associations of this sort would persist through to metaphase, and might be interpreted as polyvalents in those instances where the remaining portion of each chromosome formed chiasmata with another. Only once was such a situation observed (fig. 2*F*), although figure 2*C* is also strongly suggestive of this condition.

The sharp increase in visible fragments at first metaphase and first telophase does not mean that they have just arisen at these divisions. It is very likely they were actually formed much earlier, at the time crossing-over occurred, but were not released until these stages of meiosis had been reached. While actual chromosome breakage may occur under some conditions, the evidence is rapidly ac-

cumulating that as a rule fragments probably arise as a result of unusual crossing-over.

The decrease of fragments between first telophase and second metaphase can only be interpreted as an actual loss. Since the en-

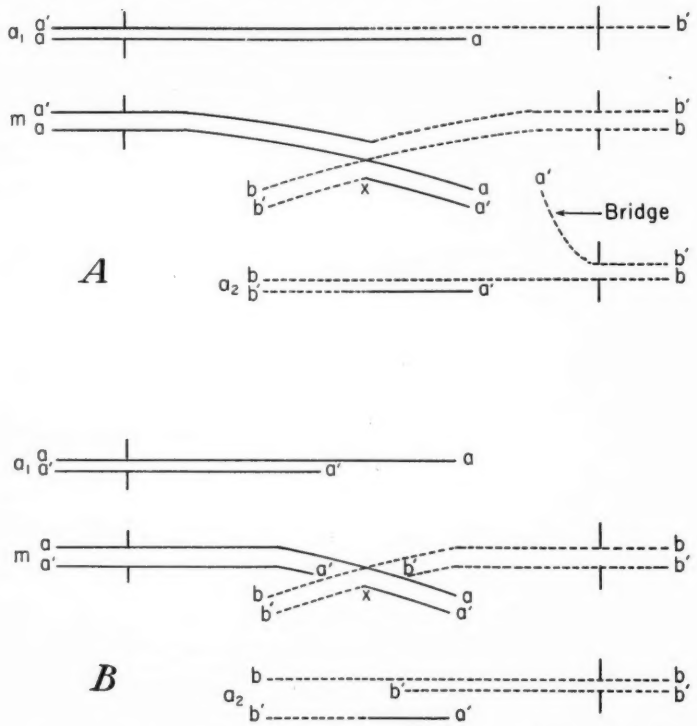

FIG. 12.—Diagrammatic details of chromatid crossing-over which results in bridge and fragment at *A*, and fragment but no bridge at *B*. Original paired chromatids shown at *m* and resulting anaphase groups at a_1 and a_2. In *B*, realignment is not as usually accepted. (A similar diagram has been used by MÜNTZING to explain possible origin of fragments in a *Crepis* hybrid.)

tire meiotic cycle takes place within the pollen mother cell wall there is no opportunity for any fragments to be cast out of the cell. In figure 5*B*, *C*, and *E* the fragments appear to be disintegrating. LEVAN (**19**) also reports the same situation in his *A. cepa*×*A.*

fistulosum hybrid, and similar observations have been reported by other investigators in other genera.

It is now generally accepted that the survival of a fragment depends on its possessing an insertion, and to a lesser degree a length sufficient to assure chiasmata formation with its homologue. Most of the fragments in this hybrid probably lack an insertion. Only a very few were observed to divide, and certainly all those resulting from formation of chromatid bridges have no insertion. While some are included in the microspores, none have been found in any of the twenty-five backcross plants which originated from pollen of the hybrid used on *A. fistulosum* and *A. cepa*. The fragments in this hybrid either persist until lost as micro-nuclei when the spore mother cell wall breaks down, or they disintegrate in the cytoplasm at earlier stages.

The atrophy and loss of some fragments, while others apparently persist, is of considerable interest. Just what happens to cause disintegration is not known. Possibly they are digested by enzymes in the cytoplasm, if they are unable to form a protective interface or chromosome membrane. In the resting cell the nuclear membrane may be considered as such an interface, which protects the chromosomes while they are undergoing metabolic changes prior to the next division. When the chromosomes contract, each appears to have formed its own membrane or interface by late prophase when the nuclear membrane disappears. The chromosomes at this time are in direct contact with the cytoplasm, and are perhaps analogous to nuclei. When a piece of chromosome is unable to form a protecting interfacial membrane it is very probably attacked and destroyed. The loss of such pieces includes, of course, the genes they carry. If the deletion is very small, the deficient chromosome may function and be transmitted to the next generation. Eventually there may arise individuals, heterozygous or homozygous for the deficiency, which because of it possess new or modified characters.

The formation of so-called chromosome bridges has now been observed cytologically in a wide variety of material. Some of these are listed in table 4. From this table it can be seen that the situation has been found in untreated as well as treated plants. Undoubtedly many earlier observations which were interpreted as non-disjunc-

TABLE 4

OCCURRENCE OF CHROMATID BRIDGES IN PLANTS

GENUS	MEIOSIS	MITOSIS	INVESTIGATOR
	Untreated species		
1. Rumex	I A	.....	Meurman, 1925 (29)
2. Ribes (3 species)	I A	.....	Meurman, 1928 (30)
3. Zea (sticky gene)	I A, II A	.....	Beadle, 1932 (2)
4. Paeonia (4 species)	I A	.....	Hicks and Stebbins, 1934 (13)
5. Matthiola (crenate)	I A	.....	Armstrong and Huskins, 1934 (1)
6. Trillium	I A, II T	.....	Smith, 1935 (38)
7. Secale	I A	.....	Lamm, 1936 (17)
8. Paeonia	I A	.....	Dark, 1936 (5)
9. Aesculus	I A	.....	Upcott, 1936 (39)
10. Tulipa	I A	.....	Woods and Bamford, 1937 (40)
11. Campanula	I A	.....	Darlington and Gairdner, 1937 (6)
12. Tradescantia (triploid)	I A, II A	Microspore	Sax, 1937 (37)
13. Lilium	.....	T	Emsweller and Brierley (unpublished)
14. Narcissus	.....	A	Emsweller (unpublished)
15. Allium (cepa)	I A	.....	Emsweller and Jones (this paper)
16. Lactuca	I A	.....	Whitaker and Jagger (in press)
	Irradiated stocks		
1. Tulipa	I A	.....	de Mol, 1930 (7)
2. Zea	I A	.....	McClintock, 1931 (28)
3. Nicotiana	I A, II A	.....	Goodspeed and Avery, 1933 (11)
4. Crocus	.....	A	Mather and Stone, 1933 (27)
5. Tulipa	.....	A	Mather and Stone, 1933 (27)
6. Vicia and Tradescantia	I A, II A	.....	Mather, 1934 (25)
7. Allium	I A, I T	.....	Levan, 1936 (22)
	Species hybrids		
1. Godetia	I A, I inter, II A	.....	Chittenden, 1928 (4)
2. Aegilops	I A, II T	.....	Miczyński, 1931 (31)
3. Nicotiana	I A	.....	Müntzing, 1934 (32)
4. Crepis	I A, II A	.....	Müntzing, 1934 (32)
5. Hemizonia	I A	.....	Clausen, 1934 (32)
6. Allium	I A	.....	Levan, 1935 (19)
7. Triticum	I A	.....	Mather, 1935 (26)
8. Pisum	I A, II A, II M	.....	Hakansson, 1936 (12)
9. Lilium	I A, II A	.....	Richardson, 1936 (36)
10. Lilium	I A, II A	.....	Ribbands, 1937 (35)
	Generic hybrids		
1. Aegilops×Triticum	I A	.....	Longley and Sando, 1930 (23)
2. Euchlaena×Zea	I A, I T	.....	Beadle, 1932 (2)
3. Triticum×Secale	.....	I T	Plotnikowa, 1932 (34)
4. Triticum×Haynaldia	I A	.....	Kihara and Nishiyama, 1937 (15)
	Exposed to low temperature		
1. Rhoeo discolor	.....	Microspore division	Dermen (unpublished)

tion or lagging univalents also belong in this list. It is interesting to note that bridges have been found in both meiosis and mitosis, in the latter also occurring both spontaneously and as a result of x-radiation.

The formation of such doubly inserted chromatids in meiosis has generally been explained as the result of crossing-over in inverted sectors. There is, however, some evidence in this hybrid that bridges also arise when inversions are probably not concerned. When two chromosomes pair in such a manner that their insertion regions are not opposite each other, and a single cross-over occurs in the interval between the insertions, a bridge will ordinarily result. As pointed out earlier, in the *cepa*×*fistulosum* hybrid such type of pairing appears to be common. In the two species, the relative location of homologous blocks of genes in two synapsed chromosomes probably causes such an association. The situation is probably brought about, therefore, more as a positional effect of homologous sectors rather than simple inversions. The single bridge and fragment observed at the first division in the *A. cepa* parent of this hybrid cannot be explained on the basis of crossing-over in inverted sectors, unless the inversion arose in a pre-meiotic mitosis. In like manner the bridges observed in mitosis as a result of x-radiation, and spontaneously in *Narcissus*, while probably arising from some sort of crossing-over, are probably not due to inverted sectors. It seems more likely that bridges arise in a number of ways from unusual crossing-over. Some of these causes may be translocations, or simple rearrangements of insertions as well as inversions.

In this material, there is also evidence that bridges do not actually break as a result of tension in the chromatid. It has become general to speak of chromosome threads, tensions, and torsions as if we were dealing with a mechanical structure. If this is the case, the chromatid forming a bridge should, following the break, move somewhat rapidly to the daughter nuclei. The fact that they do not do so is shown by frequent observations of free ends jutting into the cytoplasm after such a supposed break has occurred. In many instances these free ends also exhibit various degrees of disintegration similar to that observed in fragments. In addition, the bridges commonly show two "breaks" with an isolated fragment left out in the cyto-

plasm between the free ends of the chromatid. This has also been observed in *Lilium longiflorum* by EMSWELLER and BRIERLEY (unpublished). If the chromatid does break under tension, it is inconceivable that two simultaneous breaks could occur in such a short distance. The writers are inclined to the opinion that the same activity is probably responsible here as in the case of disintegration and loss of small fragments. When a chromatid with two insertion regions is pulled out across the cell, it finally becomes very attenuate. The resulting bridge usually persists unbroken through anaphase and usually well into telophase. At this time the daughter nuclei are being organized, the chromosomes losing their visible identity, and the formation of nuclear membranes just beginning. The attenuated chromatid is now left out in the cytoplasm where it probably attempts to go through stages comparable with the portions included in the daughter nuclei. It seems likely also that its membrane is breaking down, and it becomes highly susceptible to attack from forces in the cytoplasm. In such a way the chromatid may be destroyed at one or more places, giving rise to the "double breaks" as shown in figure 6*B* and *D*.

The occurrence of bridges in the second division of meiosis has been explained as a result of their persistence after failing to "break" at the first division. In our hybrid this probably does occur occasionally (fig. 7*D*), but it is not common and certainly cannot explain the high frequency of second division bridges. In the hybrid, as well as *A. cepa* and *A. fistulosum*, cytokinesis follows rapidly after the first division. By second metaphase the cytoplasm is well organized in two distinct bodies which may easily be separated by rupturing the pollen mother cell wall. When bridges do occasionally persist into the second division, it is probable that such cells become disorganized, as is apparently the situation in figure 7*D*. Since the evidence indicates that only a very few first division bridges persist into the second, the majority must reach this stage in some other way. As pointed out earlier, when two paired chromosomes with their insertions not opposite cross over in the region between them, there results a chromatid with two points of attachment. Similar unusual chromatids are also formed in other ways, as already noted. Since the insertions are not exactly opposite, they probably segre-

gate to the poles independently, so that both go to the same one about 50 per cent of the time, and to opposite ones with like frequency.

However, bridges of the "loop" type observed by SMITH (**38**) in *Trillium* always go to the same pole in the first division and then form a bridge at the second. He also found a second type which always formed a bridge at first division. The loop bridge was the result of a double cross-over, one within the inverted region and one proximal to it. The second bridge resulted from a single cross-over in the inverted sector. In both types the insertions were exactly opposite each other and segregated in the normal manner, one to

TABLE 5

FREQUENCY OF BRIDGES AT FIRST AND SECOND MEIOSIS

First division			Second division		
No. cells observed	No. bridges	Average per cell	No. cells observed	No. bridges	Average per cell
858	148	0.1724	229	32	0.1397

each pole. The second type was the most common and when present would show an unexpected increase in first division bridges.

A count of the relative frequency of bridges in first and second divisions in the *cepa*×*fistulosum* hybrid is shown in table 5.

If all the bridges formed in this hybrid were of the type that permitted independent segregation of the two insertion regions, we might expect to find about as many at the second as at the first division. Here, however, it must be pointed out that some of the second division bridges should be so oriented that they again go to one pole and become included in the microspore nucleus. On a purely chance basis then, there should be about half as many bridges at second as at first division. It is probable that some of the types formed in this hybrid do not appear until the second division. This would account for the unexpectedly high frequency of bridges at second division.

A comparison between the normal and one method of bridge

formation in meiosis and the first pollen grain division is shown in figure 13. If a cross-over occurs between the attachments, a bridge will be formed provided the insertion regions are oriented to opposite poles. If they go to the same pole a bridge will not be formed, but in each type a fragment will occur. In the second division the bridge chromatid in a nucleus behaves essentially the same as at the first division. If the two insertion regions of the same chromatid are oriented to different poles, a bridge results; if not, they go to the same pole and enter a microspore nucleus. The second division merely completes the separation of chromatids held together at the attachments, and there is no splitting as occurs in mitosis. Thus a second division bridge is a single structure, as shown in figure 7*C*. In the microspore the division is a normal mitosis and the chromosome with two insertion regions is split longitudinally. As pointed out by MATHER and STONE (**27**), the mitotic division of such a chromosome may result in perfect separation, or in the formation of two bridges which usually will lie across one another forming an X configuration as shown in II of figure 13. If the chromosome is oriented as at I in figure 13, there will be no bridge. If the chromatids become twisted, however, so that the insertions on each one are oriented to opposite poles, the two bridges will arise.

We did not observe any bridges in microspore divisions, but this cannot be accepted as final evidence since so few microspores in the hybrid are normal, and bridges can be identified with certainty only at anaphase and telophase. We are deeply grateful to Dr. ALBERT LEVAN, who while at the United States Horticultural Station, Beltsville, Maryland, kindly allowed us to examine some of his slides of irradiated *Allium schoenoprasum*. In this material we did find a bridge in the anaphase of the first division of a microspore. Since this material had been irradiated during early prophase, this bridge was probably formed sometime during early meiosis. Double attachment chromosomes at both metaphase and anaphase of pollen grain divisions in irradiated *Tradescantia* have been reported by MATHER (**25**). SAX (**37**) also found bridges in microspores of a triploid *T. bracteata*.

Just as this paper was completed we were fortunate in finding cytological confirmation for the figure of a microspore bridge as

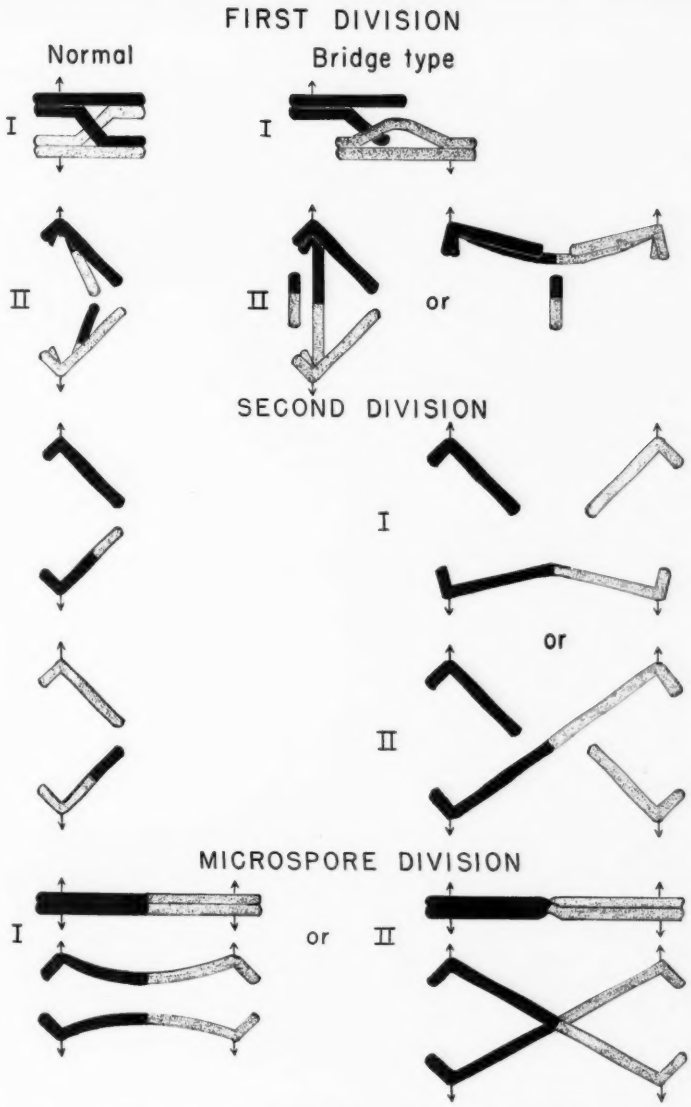

FIG. 13.—Diagram showing origin of chromatid bridges in meiosis, and their behavior through first microspore division.

shown in the diagrammatic drawing of figure 13. The photomicrograph is shown in figure 14. It is from a dividing microspore of *Rhoeo discolor* taken from a plant that had received a low temperature exposure. The plant was grown in a greenhouse at 60° to 70° F. and moved to a low temperature of 40° to 50° overnight. This slide

FIG. 14.—Dividing microspore of *Rhoeo discolor* from plant exposed to low temperature during meiosis. Note X configuration of two bridges.

is from material collected 11 days following exposure. We are grateful to Dr. HAIG DERMEN of this Division, who kindly permitted us to use this figure. This bridge is a cytological demonstration of the second type of microspore division as shown in figure 13.

If a chromosome with two insertions is included in a functional

microspore, it may eventually reach a zygote where it could conceivably persist for several cell generations. The bridges observed by PLOTNIKOWA (**34**) in derivatives of his wheat-rye hybrids were probably the result of somatic crossing-over, since single bridges and not X configurations were observed in each instance. In addition a mitotic chromosome with two insertions, even though forming two bridges, would not be accompanied by a fragment such as shown in his figures. The bridge observed by EMSWELLER in a *Narcissus* root tip was also single and was accompanied by a fragment. This was also true of those observed by EMSWELLER and BRIERLEY in somatic divisions of *Lilium*.

Discussion

The high frequency of bivalent formation in this hybrid is undoubtedly indicative of the presence of many common genes in the two species. It also indicates that crossing-over is frequent, and this is further borne out by the new chromosome types found in the first post-meiotic mitosis. Since all the hybrid microspores examined contained one or more new chromosomes, it seems very likely that some of these new forms have been transmitted to the backcross plants now in our cultures.

The formation of these new chromosomes is undoubtedly the result of the unusual crossing-over described earlier in this paper, which produced translocations, deletions, and fragmentations, and is probably responsible for the observed changes in chromosome morphology. There are also probably many other changes too small to be seen cytologically, but possible of causing new pairing conditions in subsequent generations. In addition, the formation of these new chromosomes indicates that chromosome individuality is dependent on the existence of normal pairing such as occurs in a well established balanced species. When this balance is upset, the chromosome is found to be plastic and capable of considerable change in its morphology. These changes are certain to produce irregularities in the next generation, however, and probably only very minor ones are transmitted. Thus the maintenance of chromosome morphology in a species is probably protected by the inability of structurally changed chromosomes to survive in competition with normal ones.

All of the fragments found in this hybrid are most reasonably explained as products of crossing-over. They also all appear to be a piece of but one chromatid. Under some conditions they are freed from the remainder of the chromatid as early as late pachytene, but are most frequent at first telophase. Fragments as early as pachytene have not heretofore been reported. Their appearance thus early indicates that the mechanics of crossing-over is completed at an early stage of meiosis.

The behavior of chromatid bridges at first and second division of the hybrid is very clear. In the first, each bridge is composed of three chromatids, one having two insertions and the others one each. They are ordinarily accompanied by a fragment, although it may not be present if it was released earlier and was not able to survive thus far. DARLINGTON and GAIRDNER (**6**) also report bridges without fragments in *Campanula persicifolia*, and suggest that some of the fragments are too small to be seen.

The bridges in this hybrid probably do not break but are destroyed by some cytoplasmic activity, possibly enzymatic. When this occurs, the parts left out in the cytoplasm appear to disintegrate rather than to be drawn in and incorporated in the new forming nuclei. This situation was inferred from the actually observed breakdown of bridge remnants still jutting into the cytoplasm during late anaphase, telophase, and even at interkinesis (figs. 5*B*, *D*, *E*, *F;* 6*A*, *B*, *C*, *D;* and 9*B*, *C*). There are also instances of more than one point of disintegration in the same bridge (fig. 6*B*, *D*).

The bridges of the second division are composed of a single chromatid with two insertion regions. They occur at the second chromosome division cycle following the cross-over which produced them. None of these bridges could possibly persist into a microspore, and none were ever found in well developed tetrads. That bridges do reach microspores has been demonstrated, however, and SAX (**37**) has explained their presence in triploid *Tradescantia bracteata* as being derived from dicentric chromatids broken at meiosis and rejoining at the first post-meiotic division.

Disintegration in the cytoplasm of parts of chromatids is of theoretical and probably of practical interest. The genes carried by these

bits of chromatin are lost, and the resulting deficiency may produce a profound change in the tissue descending from such a deficient cell. It seems likely that minute losses of this sort are more common than has previously been supposed. It is probable that more and more of them will be reported, since the bridge and fragment mechanism is now more clearly understood. In addition, there are probably other ways in which small fragments may arise and be lost in both meiosis and mitosis. It is not to be inferred that all spontaneous bud sports or mutations arise in this way, since reverse mutations eliminate a loss theory. It is probable that a number of causes are active, but it appears likely that spontaneous deficiencies may explain some of the many irreversible bud sports that occur with such comparatively high frequency on many horticultural plants.

Summary

1. The hybrid between *A. cepa* and *A. fistulosum* is unusual because of the high frequency of complete pairing, fragmentation, and formation of chromatin bridges.

2. Fragments were found as early as late pachytene, increasing in frequency to first telophase, then dropping. Their origin is explained as a result of unusual crossing-over. The decrease in number is probably due to an actual loss. Their possible digestion in the cytoplasm by enzymes is suggested as an explanation for this.

3. Chromatin bridges were found at first and second anaphases and telophases. It is suggested that these bridges probably arise from unusual crossing-over, some in inverted sectors, but to a considerable extent because of positional effect of insertion regions or homologous sectors. The cytological evidence also points to some interpretation other than breakage as an explanation of their dissolution. From the behavior of these bridges it was inferred that some reach the microspores, depending on the chance orientation of their two insertion regions. In the microspore mitosis they will form an X configuration when the insertion regions of one chromatid are oriented to opposite poles. This was confirmed cytologically on a slide from *Rhoeo discolor* kindly lent us by Dr. DERMEN of this Division.

4. The presence of morphologically new chromosomes was determined in the first division of hybrid microspores. These were undoubtedly formed by crossing-over between the two genoms.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES
U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

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SYNTHESIS OF FATS BY GREEN PLANTS[1]

GEORGE O. BURR AND ELMER S. MILLER

(WITH THREE FIGURES)

Literature review

In 1804, DE SAUSSURE (**22**) studied the respiratory quotients of many species (*Quercus*, *Aesculus*, *Robinia*, *Sedum*, etc.) and reported that the value of the quotients was unity. He also found a respiratory quotient of 1.0 for germinating seeds (*Vicia faba*, etc.). In 1849, REGNAULT and REISET published their experiments which showed that the value of the respiratory quotient depended on the nature of the food given to animals. By 1866 PETTENKOFER and VOIT had developed the method of measuring change in weight, respired water, and carbon dioxide, and from these figures calculating what substances had burned in the body.

BERT (**2**) seems to have been the first physiologist interested in plants to study the respiratory quotient under different circumstances and to discuss the results in relation to formation and destruction of reserve food. DEHÉRAIN and MOISSAN (**5**) concluded that the evolution of carbon dioxide and absorption of oxygen in respiration were not directly related, a view not accepted by BONNIER and MANGIN.

Although both PRESTA and DE LUCA (**14**) showed that olives could make oil after separation from the tree, GODLEWSKI (**10**) for the first time measured the respiratory quotient of ripening oily fruits. Using excised seeds of *Papaver* and *Ricinus*, he found values of 1.18 to 1.52. He discussed the effect on the respiratory quotient of chemical composition, fermentation, and change of fat to starch. BONNIER and MANGIN (**3**), in their series of papers published from 1884 to 1886, improved methods of analysis and studied the effect on respiratory quotient of temperature, oxygen tension, light, anesthetics, developmental periods, season, etc. Their studies were made on fungi, germinating seeds, and green leaves of many species. With the last they attempted separation of respiratory and photo-

[1] A contribution from the Department of Botany, University of Minnesota.

synthetic quotients. Although they recognized that the respiratory quotient was not a constant, they made no effort to use it as a measure of the nature of the metabolic process in progress.

In the same years that ATWATER and coworkers in this country and ZUNTZ and coworkers in Germany were determining exactly the respiratory quotient and calory value of animal foods, GERBER (**7, 8, 9**) in France was conducting extensive experiments in an effort to determine the nature of the metabolic process from the respiratory quotient, supplemented by tissue analysis. He followed the

TABLE 1

RESPIRATORY QUOTIENTS OF OLIVE SEEDS DURING MATURATION

DATE	COLOR OF SEED	RESPIRATORY QUOTIENT
July 15	Green olive	0.79
October 6	Color changing	1.51
October 22	Ripe olive	0.68

gas exchange of detached fruits and seeds inclosed in a respiration chamber in order to determine whether the fat was formed in the leaves and then translocated into the fruit or whether the fat was formed in the fruit from carbohydrates. His first work was with olives. It had been shown by DE LUCA (**14**) that green olives were very rich in mannite, which was replaced by oil as the olives ripened. GERBER'S respiratory quotients of olive seeds (during maturation) are summarized in table 1.

GERBER made a similar study with *Ricinus* and described the results as follows: The respiratory quotient of *Ricinus* seeds is less than unity during the very early age while they are soft. During this period the amount of glucose and sucrose is considerable, while oil is absent or present only in traces. The respiratory quotient rises above unity when the seeds have become somewhat firm. During this period the proportion of sugars decreases and the oil increases. When the seeds are hard and fully ripe, sugars are present only in traces and oil has reached a maximum value. The respiratory quotient has now fallen below unity.

GERBER concludes that his results confirm the theory of MUNTZ and of DU SABLON that fats are formed in fruits and seeds from carbohydrates.

In his later work GERBER divides the ripening of olives into four phases:

1. Transformation of mannite to oil, 4–5 days long. Respiratory quotient falls from 1.45 to 1.14.

2. Complete oxidation of a little oil, 15–30 days long. Respiratory quotient falls from unity to about 0.71.

3. Transformation of a little of the oil into carbohydrate. A short period in which respiratory quotient is 0.55–0.65.

4. Complete oxidation of carbohydrate formed in preceding period. Respiratory quotient rises to as high as 0.88 and then falls to 0.75.

From a study of temperature coefficients and surface effects, GERBER concludes that the respiratory quotient of oil formation can be distinguished from the respiratory quotient of either acid burning or ordinary fermentation.

MCCLENAHAN (**16, 17**) analyzed black walnuts at 14-day intervals during the period of rapid development. Since no starch, sugar, or tannin was detectable in the kernel at any period, he concluded that fat in the walnut is not formed from these substances and considered the possibility that fatty acids moved into the capsule. However, the evidence seems inadequate for his suggestion that fat is formed from tannin in the hull. He also reports a large percentage of water soluble material in the alcohol-ether extract during the period of rapid development and oil formation. Some of this might be a mobile lipid, but the method of extraction gives no real clue as to the nature of this material and he presents no further chemical evidence.

MCCLENAHAN made the interesting observation that the xylem was very rich in stainable fat while the phloem contained none. This might have some bearing on fat transport, although it is believed by some workers that concentration of a compound in xylem or phloem does not mean that tissue is the path of transport.

Fat formation has been studied more extensively in flax than in any other seed. IVANOV (**12, 13**) observed that during the period of

fat synthesis there was a decrease in the amount of total carbohydrate. He also concluded that saturated fatty acids were formed first and these in turn were desaturated. Later work with flax has brought out the following points. The seed develops to full size before rapid oil formation begins (6). Then in a period of about fifteen days all of the oil is laid down. The percentage of oil in the dried seed reaches a maximum within twenty-four days after flowering, but desaturation continues until full maturity (EYRE, quoted by ARMSTRONG and ALLAN 1). Lipase activity of flax seed decreases rapidly during the period of oil formation (23). The most marked decrease in enzymatic activity takes place just after the period of most rapid oil formation, the seventeenth day (24). There is no low molecular weight intermediate that accumulates to a measurable degree, since the saponification value and neutralization number of the oil are constant throughout the development of the seed.

Investigation

This paper presents a study of the value of the respiratory quotients of the seeds of castor bean, *Ricinus communis* L. var. *lividus* (Jacq.) (*R. sanguineus* Hort., *R. obermannii* Hort.), during seed development and maturation. It was our purpose to secure more complete data on gas exchange of oily seeds measured by the most accurate methods now in use. The seeds are kept attached to the plant so that normal exchange of solutes between the plant and seed will continue during the experiment.

MATERIAL.—For a study of this type, the plants should fulfil the following requirements: (a) The fruit must synthesize and store a considerable amount of fat. (b) The fruits should be capable of developing and maturing under greenhouse conditions. (c) The seeds should, preferably, be borne separately. Such an arrangement permits the removal of one or more seeds for fat analyses without materially disturbing the others. (d) The fruits and leaves should be so arranged that they may be conveniently isolated in a plant chamber. (e) The plant should be so sturdy as to permit repeated handling. These requirements were met satisfactorily by potted castor bean plants.

APPARATUS.—The apparatus employed is illustrated in figure 1. *C* is a constant speed spirometer which forces a gas stream of known composition through the metabolism chamber. *F* is a series of collecting bulbs which continuously sample the outgoing gas stream. They are full of dry mercury at the beginning of a run and are so arranged that only one is collecting gas at a time. The rate of collection of gas is controlled by the windlass which is on the same

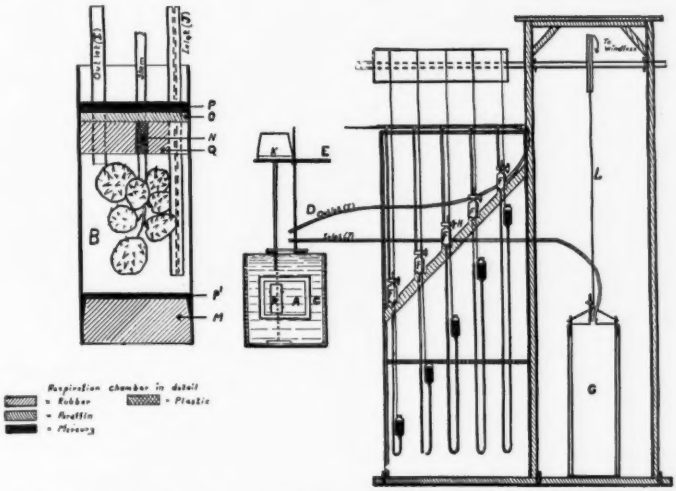

FIG. 1.—Apparatus for continuous sampling of gas stream; detailed drawing of respiration chamber at left.

shaft as the spirometer. Thus a constant fraction of the total gas stream is always being taken. The total number of samples and the period covered by each sample can be altered at will. By the insertion of capillary mercury traps above each sampler, the system becomes entirely automatic. If the air stream is dried after leaving the metabolism chamber, samples may be held without change until it is convenient to make the analyses.

A HALDANE-CARPENTER (4) apparatus was employed for the quantitative determinations of oxygen and carbon dioxide. The analyses were made in the manner described by MILLER and BURR

(19). The respiration chamber and the seal employed in this study are illustrated in detail in figure 1. The dimensions of chamber *B* are: diameter 57 mm., length 150 mm. The lower end is closed by a rubber stopper, *M*, which is covered by a layer of mercury, *P*. About 40 mm. from the other end, a split stopper, *Q*, is inserted. The space between the stopper and seed stalk is filled with a plastic, *N*. A layer of low melting paraffin is poured above the stopper, care being taken that the temperature of the paraffin does not exceed 45° C. After the paraffin has solidified, it is covered by a layer of mercury, *F*. This type of seal does not injure the seed stalk. As long as a layer of mercury remained at each end of the chamber, no leaks could be detected. The distance that the cut stopper is inserted into chamber *B* depends on the size of the cluster of castor bean seeds. In all the experiments the volume of chamber *B* is kept as small as possible, 150 cc. or less. The diameter of the inlet tube, *J*, is 1 mm. and of the outlet tube, *I*, 6 mm. The chamber is immersed in a water bath which is maintained at a temperature of 28°–29° C. by a heater and thermostat.

Diffusion of carbon dioxide and oxygen through castor bean petioles

Before the study of the gaseous exchange of different organs was undertaken, the rate of diffusion of carbon dioxide and oxygen through castor bean petioles was investigated. In all the following experiments, the seal illustrated in figure 1 was employed.

In experiments 1 to 4, the plants were brought into the laboratory twelve hours before the diffusion experiments were begun. In these experiments the leaves were inclosed in 3-liter Erlenmeyer flasks. As an aliquot portion of gas was removed, an equal amount of water was introduced into the respective flasks in order to maintain the same pressure around the inclosed leaves as that surrounding the rest of the plant.

Before carbon dioxide was introduced into the flasks, the rate of respiration was determined over a 2-hour period. Carbon dioxide was introduced into the leaf chamber and analyses were made at the beginning and at the end of a 2-hour period. After the carbon dioxide had been replaced by air, the rate of respiration was again

determined for another 2-hour period. From the average rate of respiration for these two periods, the rate of respiration was calculated for the period when the leaf was in an atmosphere containing approximately 1 per cent carbon dioxide.

The data presented in table 2, experiment 2, may be used to illustrate the calculations of the values in the columns under "calculated" and "observed." A leaf was inclosed in the respiration chamber containing room air (0. 036 per cent carbon dioxide), and the rate of respiration was determined for three hours. During this period the leaf respired 7.027 mg., or 2.342 mg. per hour. Carbon dioxide

TABLE 2

DIFFUSION OF CARBON DIOXIDE THROUGH CASTOR BEAN PETIOLES

EXPERIMENT	DURATION (HOURS)	RESPIRATION PER HOUR (MG.)	OBSERVED MG. CARBON DIOXIDE	CALCULATED MG. CARBON DIOXIDE	PERCENTAGE DIFFERENCE
1	1.33	0.19	53.40	53.60	−0.4
2	3.00	2.42	61.21	61.18	+0.05
3	2.92	1.19	53.15	53.10	+0.1
4	3.83	2.96	11.63	11.44	+1.7

was introduced into the respiration chamber, and the analyses of the aliquot portion showed that the atmosphere contained 53.924 mg. Three hours later the atmosphere contained 61.218 mg. The respiration chamber was now opened and permitted to come to equilibrium with room air. The rate of respiration was again measured and found to have increased the carbon dioxide concentration of the atmosphere by 7.495 mg., or 2.498 mg. per hour. The average of the rate of respiration of the first and last period was employed in calculating the amount of respiration which occurred during the period that the leaf was in an atmosphere enriched with carbon dioxide. This correction was 7.261 mg., which must be added to 53.924 mg. Hence the caculated value was 61.185 mg., as shown in the table. It was assumed that the higher concentrations of carbon dioxide had no effect on the rate of respiration.

In view of the fact that a different plant was used in each of the preceding experiments, the results indicated in table 2 are in excellent agreement. The error recorded is approximately equal to the

total possible analytical error of the three carbon dioxide determinations by the HALDANE-CARPENTER apparatus. It would appear, therefore, that with a carbon dioxide concentration difference of about 1 per cent, no appreciable loss takes place by diffusion through the petiole.

A few experiments were made to determine the rate of diffusion of oxygen through castor bean petioles. The data summarized in table 3 were obtained by the same type of calculations as that described for carbon dioxide.

The rate of oxygen consumption by leaf respiration was determined for an initial period. Then the atmosphere in the respiration

TABLE 3

DIFFUSION OF OXYGEN THROUGH CASTOR BEAN PETIOLES

EXPERIMENT	DURATION (HOURS)	RESPIRATION PER HOUR (MG.)	OBSERVED MG. OXYGEN	CALCULATED MG. OXYGEN	PERCENTAGE DIFFERENCE
21	2.0	0.888	21.40	20.30	+5.1
22	2.0	1.172	16.06	16.36	−1.8
23	2.0	1.134	13.92	13.72	+1.4
24	3.0	0.888	19.86	18.74	+5.6

chamber was enriched in oxygen by approximately the amount recorded in the column under "observed." In these experiments it was necessary to assume that increasing the oxygen content of the atmosphere by approximately 0.5 per cent had no effect on the rate of respiration.

The maximum analytical error for oxygen determination (±0.005 per cent) is so great that in a series of analyses on such small samples, the summed error may become 5–10 per cent of the total respiration; therefore these figures do not have great exactness. The differences found are within the experimental error, and since these are usually positive, there is a strong indication that with a 0.5 per cent gradient, oxygen does not diffuse at an appreciable rate through leaf petioles.

MEASUREMENT OF RESPIRATORY QUOTIENTS OF SEEDS

After the plant had remained one hour in the laboratory, the seeds were inclosed in the respiration chamber. The apparatus was run for

two hours before the collection of samples was begun. A sample was collected every five or six hours during each run.

In this study, the respiratory quotients were determined at 6 to 9-day intervals after fertilization of the ovule. Following each experiment, one seed ball was removed from the plant for quantitative analysis of the total fat content. The seeds were dried in a vacuum oven at 40° C. to a constant weight, and then stored in an atmosphere of carbon dioxide until analyzed. The A.O.A.C. method (20) was employed for determination of the total fat content.

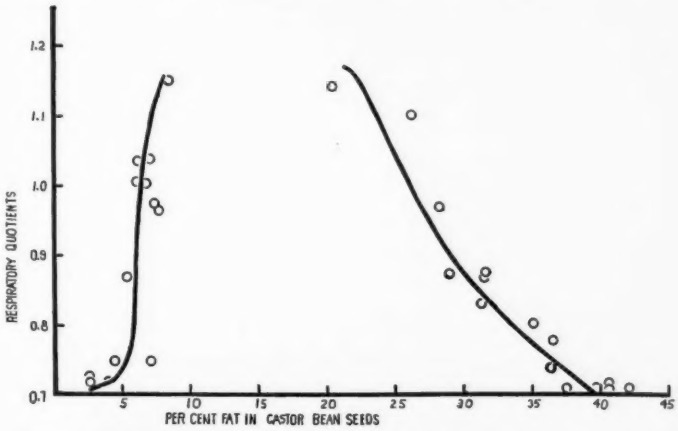

FIG. 2.—Respiratory quotient of castor beans plotted against oil content. Most of the oil appears during a few days of high respiratory quotient.

The results of these determinations are presented in figures 2 and 3. The first measurements (shown by the circles in the lower left hand corner) were made approximately seven days after fertilization. During this early period, the quotients remained below 0.75 and the total fat was between 3 and 6 per cent of the dry weight. During the next fifteen days there was a gradual increase in respiratory quotient with very little increase in fat. It was only after the respiratory quotient had risen above 1.00 that rapid accumulation of fat occurred. However, there was an appreciable increase in fat after the respiratory quotient had again fallen below 1.00 and even below 0.8.

These data confirm the observations of GERBER (**7, 8, 9**) and GODLEWSKI (**10**) that the respiratory quotient is greater than 1.00 during the period when fat accumulation is at a maximum. These high quotients are in accord with the view that considerable fat synthesis occurs in the seeds from sugars brought in from the leaves. They do not exclude the possibility, however, that some fat is synthesized in other tissues and then transported to the seeds.

In fact, a slow accumulation of fat takes place during the very early period and again during the final ripening period when the

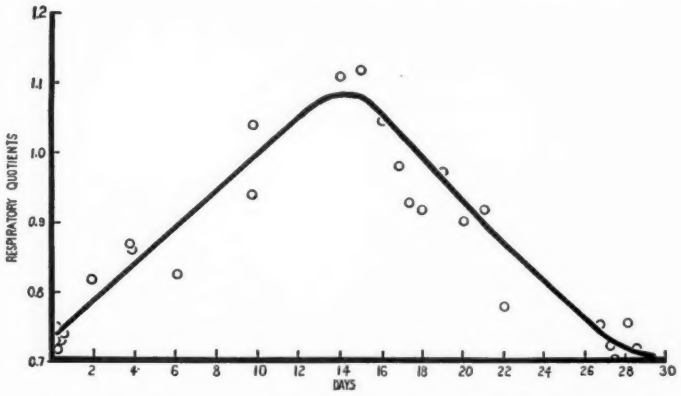

FIG. 3.—Respiratory quotient of castor beans at various stages of maturity

quotients are well below unity. These data indicate an inward movement of lipid. However, such an interpretation loses much of its argument in view of the fact that GERBER found low quotients with detached fruits.

That the leaf is capable of furnishing a ready supply of lipid material is indicated by the extensive work of MAQUENNE and DEMOUSSY (**15**). Using improved methods, they found high and fairly uniform values for respiratory quotients of all leaves. An average value of 1.046 is given for hundreds of determinations on forty species whose leaves were fresh and active (**15**, p. 111). Such a value indicates that the average leaf is actively producing fatty acids, alcohols, hydrocarbons, or other compounds containing little

oxygen as long as it is well supplied with carbohydrates. Some of this lipid material might be translocated. A young active leaf, when kept at high temperatures, may so very quickly deplete its store of carbohydrates that after a few hours in darkness its quotient has fallen to 0.8–0.9 (**15**, pp. 42–43).

A respiratory quotient of 0.7 indicates a use of pure fatty acids or some hydrocarbons or alcohols as fuel for respiration. However, since fat is slowly accumulating, its use in respiration would require that even larger amounts be moved in from the rest of the plant. On the other hand, if carbohydrates chiefly are being burned, this process must be accompanied by the synthesis of compounds of still higher oxygen content. Acids such as malic, tartaric, oxalic, citric, glucuronic, and galacturonic are richer in oxygen than glucose, and their formation could produce quotients as low as 0.7 provided several molecules of acid were formed for each molecule of glucose burned to carbon dioxide and water. For example, if glucuronic acid were being formed for the production of gums and hemicelluloses, three molecules of this acid would be required for each molecule of glucose burned to give a quotient of 0.67. This quotient is low enough to mask slow fat synthesis when the observed (composite) respiratory quotient is between 0.7 and 0.8.

RHINE (**21**) was confronted with a similar difficulty in interpretation of respiratory quotients. Working with both oily and starchy seeds he found the respiration quotient of the hypocotyl to be about 0.77. This might indicate that fats were being transported to the growing tip and burned. However, by analysis he found a concentration gradient in the opposite direction and concluded that the movement of oils as such is improbable. It is well to remember that gradients calculated from total ether extract of tissues are under suspicion since a large portion of total lipids in active cells may be combined and non-mobile. MILLER (**18**) found that during the last week of germination the free acids were nearly all of low molecular weight and soluble in water. These fatty acids are mobile, undetectable by staining methods, give low respiratory quotients when burned, and could be made into higher fatty acids with little effect on gas exchange ratios.

A paper by GUSTAFSON (**11**) has just been read. In tomatoes dur-

ing ripening he finds a rise in respiratory quotient to a value above unity, which is thought to be caused by a shift in carbohydrate-acid metabolism. He gives some interesting speculation on the meaning of the quotients found, but concludes that further analytical data are required for adequate interpretation of results.

Summary

1. An apparatus is described which permits aliquot parts of gas streams to be collected from the respiration chamber at desired intervals.

2. A method is described in detail for making a seal to inclose an attached leaf or fruit in the respiratory chamber, without apparent injury to the plant.

3. Under the described experimental conditions, no diffusion of either carbon dioxide or oxygen through petioles could be detected.

4. Data presented show that a considerable quantity of fat is synthesized within the castor bean fruit.

5. The possibility of translocation of some fat is discussed, and it is concluded that more detailed analytical data are required for determining whether some type of lipid is slowly moved into castor bean fruits during periods of low respiration quotients.

DEPARTMENT OF BOTANY
UNIVERSITY OF MINNESOTA
MINNEAPOLIS, MINNESOTA

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DEVELOPMENTAL ANATOMY OF THE SEEDLING OF THE RICE PLANT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 490

CHI-TUNG YUNG[1]

(WITH THIRTEEN FIGURES)

Introduction

Although the rice plant (*Oryza sativa* Linnaeus) has been investigated extensively by geneticists, physiologists, and taxonomists, comparatively little attention has been given to its morphology. BRUNS' work (6) on the grass embryo includes a brief description on the rice embryo and its development. HAAN (8) made an anatomical study on the various parts of the mature plant and the grain. The flower and caryopsis were investigated by AKEMINE (1), WEATHERWAX (15), and SANTOS (13). NOGUCHI (10) studied the cytology of the megagametophyte, fertilization, and the young embryo. The present study endeavored to cover the seedling stages.

Studies on the morphology of the grass embryo and seedling have been intensive during the past century. Various writers have had divergent interpretation of the homologies of the different structures in the embryo. Excellent reviews of the literature dealing with this subject have been given by BRUNS (6), WORSDELL (16), AVERY (3), and others. The more recent publications of ARBER (2), MCCALL (9), REZNIK (11), and BOYD and AVERY (5) indicate that the controversy is still far from being settled. The chief argument is centered on the morphological interpretation of the epiblast and the relation between the scutellum and the coleoptile.

Material and methods

Two varieties of rice were used in this investigation, the Fortuna and the Caloro. They were obtained from the United States Department of Agriculture, Bureau of Plant Industry. Grains were

[1] Research Fellow of the China Foundation for the Promotion of Education and Culture.

soaked overnight for the study of the embryo. For seedlings, grains were grown in damp soil in the greenhouse and in water culture. No structural difference has been found in the two lots of seedlings. The materials were fixed in CROOKS' modification (7) of Navashin's fluid, and imbedded in paraffin. Serial sections were cut at 7–12 μ.

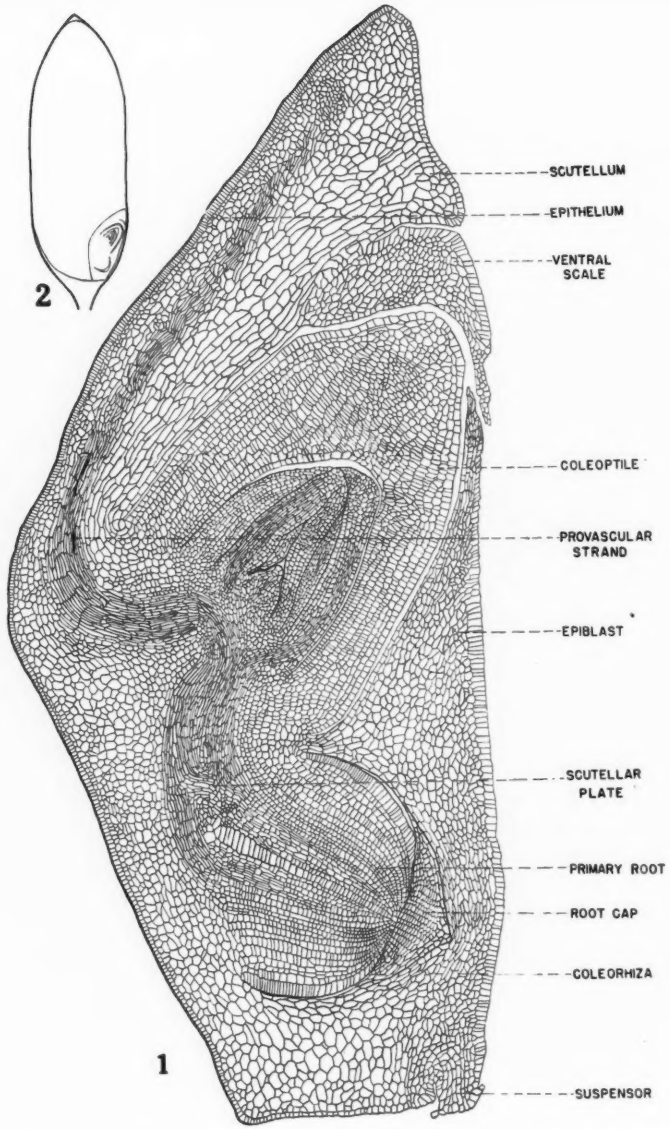
Investigation

EMBRYO.—The embryo lies at the base of the grain on one side of the endosperm, in a slanting position (figs. 1, 2). In longitudinal section it has the shape of a semi-pentagon, two of its sides being in contact with the endosperm. In cross section it is spindle-shaped. The central axis of the stem and the root is conspicuously curved. The elongated cotyledon lies above the epicotyl, with an outgrowth from its under side, the ventral scale. The cone-shaped epicotyl is enveloped by the coleoptile. The hypocotyl, consisting of the primary root and the coleorhiza, occupies the basal part of the embryo. A large epiblast is present approximately at the level of divergence of the cotyledon. Its lower end is continuous with the coleorhiza. The upper free end extends near to the top of the coleoptile and is overlapped by the overhanging ventral scale. The latter and the epiblast inclose the coleoptile.

The cotyledon or scutellum consists chiefly of parenchymatous cells. Its surface next to the endosperm consists of a conspicuous layer of rectangular epithelial cells (fig. 1). Epithelial glands, or the infoldings of the epithelial layer, are not found. Cells of the ventral scale, epiblast, and coleorhiza, although smaller in size, are similar to those making up the bulk of the cotyledon.

The apex of the stem axis has a small growing point which together with the embryonic leaves is completely inclosed by the cone-shaped coleoptile. Near the tip of the latter there is a slitlike opening. The cells of the coleoptile and the embryonic leaves are regularly arranged in tiers. The cells at and slightly below the divergence of the coleoptilar bundle are highly meristematic; their activity during germination results in the elongation of the first internode.

The primary root is the only seminal root present in the resting embryo. It lies approximately at a right angle to the axis of the



FIGS. 1, 2.—Fig. 1, median longitudinal-side section of embryo. Fig. 2, same, of caryopsis, showing location of embryo.

epicotyl. The apical meristems are protected by the root cap, which in turn is enveloped by the coleorhiza (fig. 1). HAAN (**8**) described the histogens of the rice root as being the type commonly found among Gramineae. Three histogens are present. The distal one is the calyptogen; the middle layer functions as a dermatogen and also gives rise to the periblem; and the upper layer gives rise to the plerome. Such a differentiation has been attained already in the embryo. A cross section made at a higher level of the root shows a differentiated epidermis and exodermis. Cells of the inner cortex are regularly arranged radially. Endodermis and pericycle are also well defined by their arrangement as single cell layers. None of these cells has lost its cytoplasmic contents, however, nor has the wall thickened.

The provascular tissue of the embryo is a continuous strand occupying the central part of the curved axis (fig. 1). The upper and lower free ends supply the scutellum and the primary root respectively. The axis of the epicotyl contains the stelar bundle, about half of which is continuous with the cotyledonary bundle. The latter diverges abruptly from the main strand near the coleoptilar node (second node), turns downward in the cortex to form the cortical bundle, and supplies the scutellum. In a cross section at the middle of the epicotyledonary axis, therefore, two bundles are found, the stelar and the cortical.

In the embryo the first internode is very short, having the same cellular structure as the upper part of the first node. As the cortical bundle bears away from the stelar bundle there arises from it a pair of coleoptilar bundles, one on each side and at right angles to the W-shaped main provascular system. Each bundle extends first laterally for a short distance and then turns upward nearly to the tip of the coleoptile. The provascular strand to the first foliage leaf diverges from the stelar bundle also at the same level. It constitutes the midrib of the first foliage leaf and lies in the same plane as the main system; that is, at a right angle to the coleoptilar bundles.

The provascular system for the midrib of the second foliage leaf is also present. At a level slightly higher than that of the divergence of the coleoptilar bundles the basal end of the strand forks into two branches; one is united with the left and the other with the right

side of the stelar bundle at the point where the cortical bundle diverges.

The provascular system in the embryonic primary root consists of a central row of parenchyma surrounded by six or seven alternating strands of immature xylem and phloem cells. These cells are distinguishable by their size and staining reaction. The central parenchyma becomes a row of vessel segments during germination.

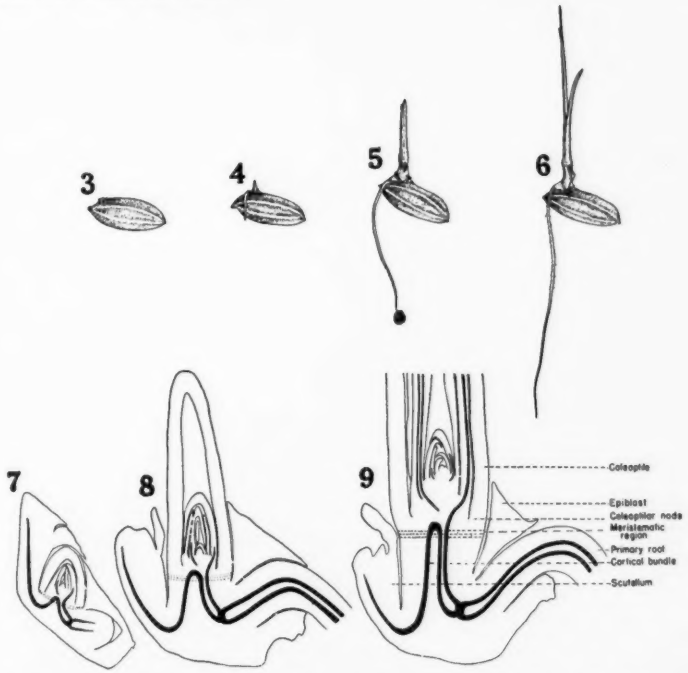
There is a vascular plate connecting the strand of the primary root with the stelar bundle. It is at this point that an abrupt root-stem transition takes place. The central row of large parenchyma branches here into two rows. Gaps filled with smaller parenchyma are found between the anastomoses of the various provascular strands forming the pith. The plate is clearly shown in the longitudinal section (fig. 2).

The cotyledonary bundle branches into small strands at the upper distal end of the main trunk. These branchlets extend backward for a short distance toward the base of the cotyledon. The lower portion of the main cotyledonary bundle does not branch at all. There is no provascular tissue in the ventral scale nor in the epiblast.

Occasionally some of the cells of the provascular strands in the embryo have secondary spiral thickenings.

GERMINATION.—Germination of the rice grain (figs. 3–9) is very much like that of the oat. Under favorable conditions the coleorhiza pushes through the pericarp, leaving a cavity in front of the root cap. The primary root soon elongates and fills the cavity. Subsequently the coleorhiza is penetrated. Upon further growth the root extends upward toward the epicotyl for a short distance before it responds positively to the stimulus of gravity. About this time the coleoptile also emerges and rapidly elongates. The cells of the meristematic region just below the divergence of the cortical bundle divide actively. As a result the part of the epicotyl below this divergence with its vascular bundles becomes greatly lengthened (figs. 7–9). The extent of this elongation varies according to the depth the grain is planted. When the base of the coleoptile has been brought to the upper level of the soil, the opening at its side opposite the scutellum splits more widely and subsequent foliage leaves soon appear.

In about two days primordia for two adventitious roots begin to differentiate in the meristematic zone on the side opposite the scutellum. They soon penetrate through the cortical region. A third adventitious root may appear later at the coleoptilar divergence opposite the two older ones. Lateral roots soon develop from the primary and adventitious roots.



FIGS. 3–9.—Figs. 3–6, stages of development in germination. Fig. 7, median longitudinal-side section of dormant embryo. Region in which first axial elongation will begin shown in dashes. Fig. 8, germinating embryo, 48 hours old, showing beginning of germination. Fig. 9, same, 4 days old.

SEEDLING AXIS.—HAAN (8) studied the vegetative structures as well as the spikelet and mature grain of rice. His description of the root, stem, and leaf was based chiefly on mature plants. He has not mentioned the vascular system, especially that of the seedling stages. A brief description of the various organs in addition to a

detailed account of the vascular skeleton of the young rice plant therefore seems desirable.

All roots, primary, adventitious, and secondary, generally are hexarch radial protostele. Roots with seven xylem strands are not infrequently found. The epidermis consists of a single layer of cells. Immediately beneath this region there is a layer of collenchyma forming the exodermis. The cortex is the most prominent part of the root; the outer one or two layers of cells have their walls greatly thickened. The large inner parenchymatous cells are filled with starch grains. The endodermis is well defined by the thickened inner tangential wall. The pericycle consists of one layer of cells.

The part of the axis between the scutellar and coleoptilar divergence in the grasses is termed the "first internode" by BOYD and AVERY (3, 4, 5). This region in rice is well marked and elongates appreciably during germination. The homology of this organ will be discussed later. Cells of the first internode are not so elaborately differentiated as are those of the root. The epidermis is present as a single layer of cells. Exodermis is wanting. The cortex consists of a wide strip of thin walled parenchyma. Imbedded in the cortex on the side next to the scutellum is the cortical bundle which runs downward and backward to the scutellum. In older seedlings air spaces are present in the cortex. The endodermis and pericycle are single layers of thin walled cells. The vascular tissue in the stele resembles that of *Avena* (3). It consists of four bundles. Two large endarch bundles are separated by a small patch of parenchyma which may be termed the pith (fig. 11*C–D*). One of the endarch bundles is a direct continuation of the cortical bundle. It is termed the "common bunble" by BOYD and AVERY (5) because of its relation with the coleoptilar bundles and the lateral bundles of the first foliage leaf. The other endarch bundle runs directly into the midrib of the first foliage leaf. The arrangement of the xylem elements in the lateral bundles is neither endarch nor exarch. In young seedlings their identity is lost at the meristematic zone just below the coleoptilar node. It is also at this level that the first two adventitious roots arise.

At the scutellar plate (fig. 11*D–F*) each of the two endarch bundles of the first internode is formed by fusion of two adjacent bundles of the hexarch stele of the primary root. Each of the remaining

unfused bundles of the root has two side branches, one on each side, which are joined to the two endarch bundles; these extend upward as the two lateral bundles of the first internode. In this manner the six bundles of the root are reduced to four in the first internode. The vascular plexus is made more complex by the fact that it occurs at the notch where the vascular skeleton of the root and stem form

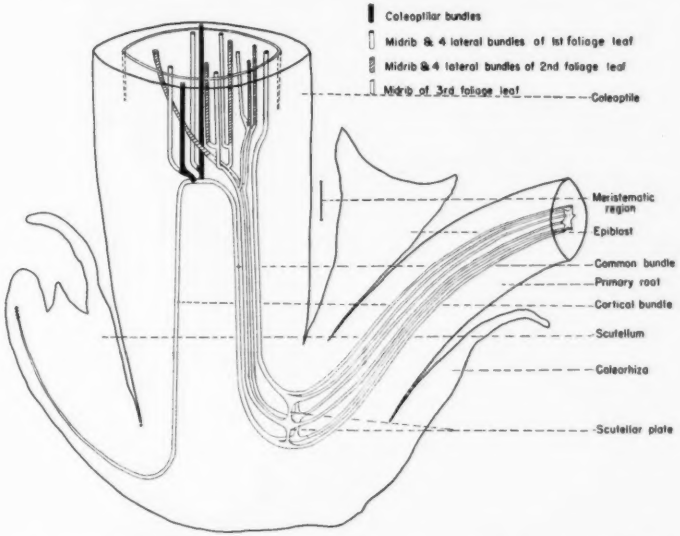


FIG. 10.—Diagrammatic reconstruction of lower portion of seedling showing principal structures present, and vascular interrelations of scutellum with axis, coleoptile, and first foliage leaves.

an acute angle. When seven xylem groups are present in the root, one of the endarch bundles is anastomosed with three instead of with two bundles, as in the case of *Avena*.

At the level of the coleoptilar divergence the following main vascular bundles are found (figs. 10, 11*B*): (1) a pair of coleoptilar bundles; (2) a midrib and two pairs of lateral bundles for the first foliage leaf; (3) a midrib and two pairs of lateral bundles for the second foliage leaf; (4) a midrib for the third foliage leaf. The relation between these bundles and the four of the first internode is as follows.

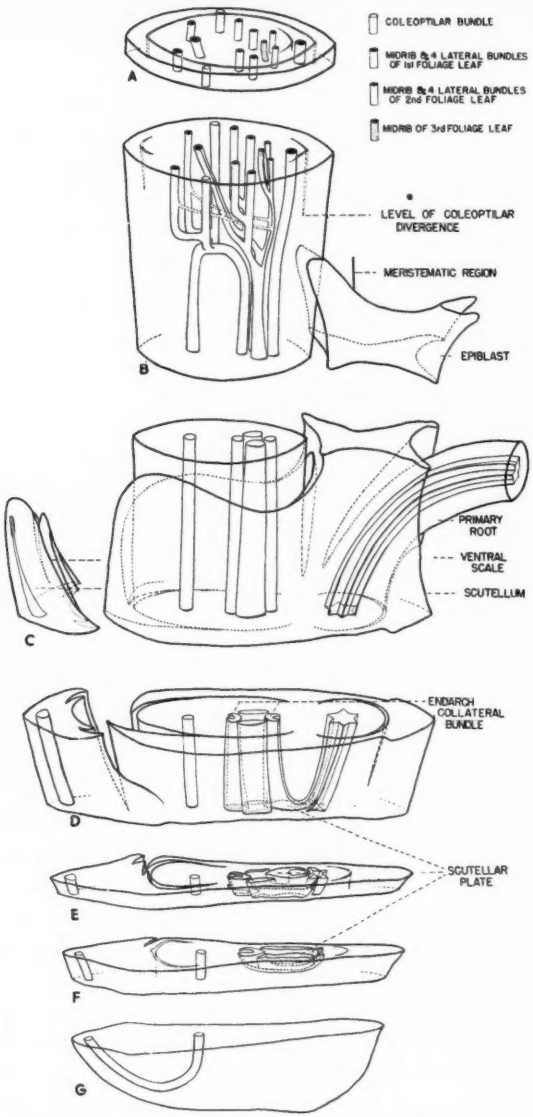


FIG. 11.—Three-dimensional diagrammatic presentation of fig. 10, showing detailed course of vascular bundles and various organs of seedling.

(1) Each of the two coleoptilar bundles is fused with an outer lateral bundle of the first foliage leaf which lies on its scutellar side and then joins the cortical bundle at the point where the latter bears away from the common bundle of the first internode.

(2) The midrib of the first foliage leaf is a direct continuation, without branching, of the endarch bundle opposite the common bundle.

(3) Each inner lateral bundle of the first foliage leaf is fused with the adjacent lateral bundle of the second foliage leaf and then with half of the midrib of the first foliage leaf. The resulting bundles then turn downward and meet the lateral bundles of the first internode at the meristematic zone.

(4) Each of the remaining two lateral bundles of the second foliage leaf is fused with half of the midrib of the third foliage leaf before they join the lateral bundles of the first internode at the meristematic zone.

(5) There is a vascular anastomosis at the second node, linking the lateral bundles of the first and second foliage with the midrib of the second foliage leaf (fig. 11*B*).

COLEOPTILE.—The point of divergence of the coleoptile and the origin of its two vascular bundles have been described. It consists chiefly of closely arranged parenchyma. In cross section cells of the outer epidermal layer are smaller in size and slightly rectangular in shape. Stomata are present, especially near the apex. Under very moist conditions exudation takes place in these pores. Few chloroplasts are present. In all the specimens studied, no coleoptile has been found to possess more than two vascular bundles. The bundles are placed approximately opposite each other, in a plane at right angles to the midribs of the later foliage leaves. Each bundle in cross section consists of a row or two of sclerenchymatous cells on the outer side. Phloem cells occupy a little more than half of the total bundle area. Xylem cells are relatively few and are arranged in a row adjacent to the inner side of the phloem. A bud primordium is present in the axil of the coleoptile (the side toward the scutellum). Rarely this primordium develops.

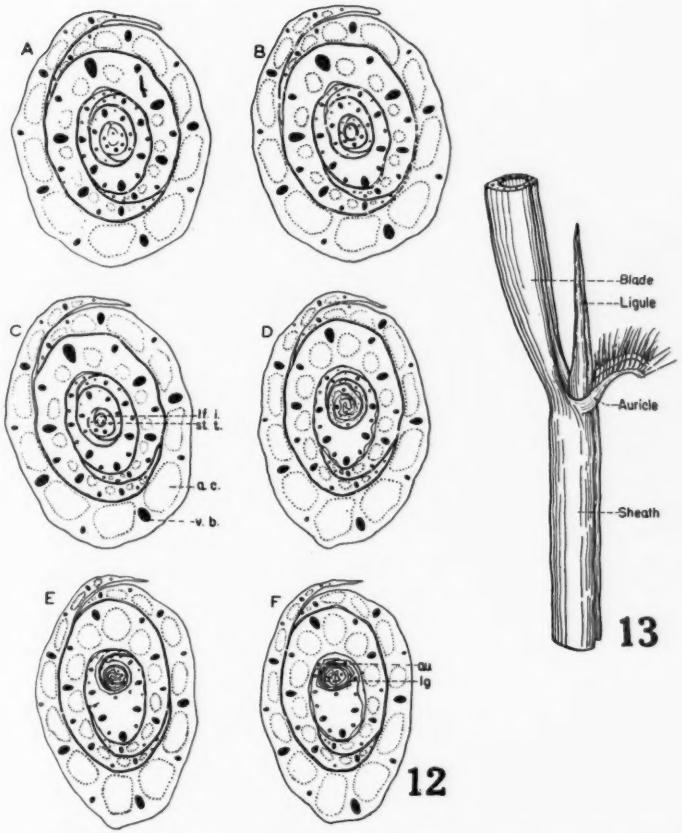
FOLIAGE LEAVES.—A mature foliage leaf consists of the sheath at the base which surrounds the culm for some distance, the blade

which is set at an angle with the culm, the ligule, and the auricles (fig. 13). Descriptions of these structures are given by other workers (**2, 8**).

RÖSELER (**12**) reported that in wheat the leaves originate from the dermatogen alone. This may also be true for rice, but the evidence cannot be considered conclusive until further investigation is made. The leaf primordium begins to develop at a point near the growing tip of the stem on the side opposite the midrib of the preceding leaf (fig. 12*C*). Soon cells at either side of it are differentiated from the stem in similar manner until the two edges of the primordium overlap, thus inclosing the growing stem tip. After two or three tangential divisions of the initial cells, a dermatogen (tunica) is differentiated. This is followed by the differentiation of the provascular tissue, first the midrib and then the lateral bundles. The primordium increases rapidly in length, and soon the sheath is visibly distinguishable from the blade. In the early stages of leaf formation the blade grows much faster than the sheath. Frequently the blade has attained almost full length while the sheath is only an inch or two in height.

There is a radial thickening in the sheath around the central leaf axis beginning at the point just above the growing tip of the stem (fig. 11*D–F*). This thickening is the result of the repeated tangential divisions of a row of cells under the inner epidermis in the direction of the midrib. The leaf is thickest at the base of the blade (fig. 12*F*). In the blade the thickening decreases gradually, and finally appears as a ridge containing the midrib and projecting from the abaxial surface of the leaf.

The ligule develops on the adaxial face of the leaf while the latter is still young and the cells are still meristematic (fig. 12*E–F*). The upper margins of the sheath are continuous with the overlapping margins of the ligule. The median portion of the ligule is an outgrowth from the adaxial epidermis of the leaf. The development of the ligule begins by a tangential division of some adaxial epidermal cells at the apex of the sheath. The new daughter cells soon split from the sheath. The splitting gradually extends laterally toward the abaxial surface of the sheath, thus incorporating the newly formed outgrowth with the inner border of the sheath to form the



FIGS. 12, 13.—Fig. 12, cross sections of epicotyledonary axis: *A*, near stem tip, showing four encircling sheaths (fifth one not yet separated from stem); *B*, 12 μ above *A*; *C*, 24 μ above *B*, showing leaf initial (*lf.i*) being differentiated from stem tip (*st.t*); *D*, 1.6 mm. above *C*, showing blade surrounded by three sheaths; *E*, 1.1 mm. above *D*, showing lower end of ligule; *F*, 0.12 mm. above *E*, showing ligule (*lg*) completely detached from leaf proper and upper end of auricles (*au*). Fig. 13, middle portion of leaf.

ligule (fig. 12*E*). In a fullgrown leaf the ligule is in a plane continuous with the axis of the sheath, while the blade is at an angle to this axis. The auricles are developed at a slightly higher level than the ligule. They first appear as two knoblike outgrowths from the base of the blade, one on each side of the lamina. Upon further growth they increase in length and long hairs develop on their edges. Where the blade bears away from the sheath, the auricles bend slightly in the opposite direction and encircle the culm.

There are small cross veins connecting the parallel bundles of the leaf sheath and blade. These veinlets begin to differentiate while all the cells are still meristematic. At more or less regular intervals single rows of cells between two adjacent bundles begin to divide. Each cell divides tangentially two or three times. Usually the cross walls are not formed until the entire series of divisions is completed. The result is that each original mother cell gives rise to a bundle of elongated cells, and these long daughter cells are joined end to end with those of the adjacent cell. In cross sections of young leaves, such chains of provascular tissues are plainly seen linking up the parallel veins.

The presence of air cavities in the leaf is characteristic of the rice plant. These cavities are large and more or less cylindrical with thin diaphragms as cross partitions. In the diaphragm small cross veins are invariably found, often forming an anastomosis. The formation of these cavities begins when the cross veins start to differentiate. Groups of cells in the leaf at regular intervals very close to each other cease to become meristematic. The cell contents disintegrate and the cell walls lose their elasticity. As the leaf continues to elongate by division and enlargement of the other cells, these degenerate ones are pulled apart, forming cavities between the living cells; the result is like a honey-comb structure in the leaf. These cavities continue to enlarge as long as elongation of the leaf takes place.

Generally, as soon as the formation of an air cavity is started, all cells except those near the base of the leaf sheath constituting the intercalary meristem begin to lose their capacity of cell division. They enlarge only in size. The provascular tissue matures into xylem and phloem elements. Sclerenchyma is differentiated. The intercalary meristem continues to give rise to new cells, adding length to

the sheath until the latter is almost as long as the blade. The new cells thus derived repeat the processes of tissue differentiation and maturation as already described.

Discussion

The rice embryo closely resembles that of the oat. They both have similar arrangement of the provascular system and general structural makeup. The position of the meristematic zones is identical in both embryos. Some points of difference are: (1) the stem-root axis of the rice embryo forms a sharp angle, whereas the axis of oat is a straight line; (2) the rice embryo has a larger epiblast and is overlapped by the ventral scale, thus completely inclosing the coleoptile; (3) seminal roots are absent where the epiblast diverges, a fact which may be related to the curved nature of the stem-root axis.

The vascular system of the rice seedling also agrees with that of the oat seedling. Both possess the inverted cotyledonary trace which runs backward and downward in the cortex all the way through the first internode. In the rice seedling each of the coleoptilar bundles is united with only one lateral bundle of the first foliage leaf, but each coleoptilar bundle of the oat is united with two lateral bundles of the leaf above. This minor difference does not mar the close resemblance.

ARBER (**14, 2**) and earlier investigators have advanced a hypothesis in an attempt to homologize the scutellum of grasses with the blade of a foliage leaf, the coleoptile its ligule, and the intervening axis, "mesocotyl," as an elongated cotyledonary node. This theory assumes that the scutellum has a closer vascular relationship with the coleoptile than with the later foliage leaves. BOYD and AVERY (**5**) have shown that such an assumption is not in accord with the situation found in the oat. The rice seedling is similar to that of the oat. The common bundle in the first internode (ARBER's mesocotyl) supplies two lateral bundles of the first foliage leaf as well as the coleoptilar and scutellar bundles. BOYD and AVERY cite the case of *Billbergia zebrina* and four other genera of Zingiberaceae to substantiate their contention that the scutellum and the coleoptile are separate organs, one being the cotyledon and the other the sec-

ond leaf. The cotyledon of *B. zebrina* has a well developed ligule, the divergence of which is far below the vascular fusion of the cotyledonary trace and the bundles of the first foliage leaf (equivalent to the part of the axis just above the meristematic zone in oat and rice). In no way can the first internode be interpreted as an elongated node. In view of this strong evidence, the writer feels justified in accepting BOYD and AVERY'S interpretation and applying the same to the findings in rice; that is, the scutellum is the first (seed) leaf, the coleoptile the second leaf, the first foliage leaf the third leaf, and so forth. Other arguments for and against this interpretation have been fully discussed by others (**2, 3, 4, 5, 6, 9, 14, 16**).

MCCALL (**9**) recently revived the old controversy that the epiblast is a second cotyledon. In his study of the wheat seedling he found a vascular anastomosis which he considered as an extra node in the axis between the scutellar and the coleoptilar divergence (first internode of AVERY). In the rice seedling, as in the case of oat, this vascular anastomosis is situated far above the scutellar divergence and has no relation to the scutellum. While the absence of vascular tissue in the epiblast is only a negative evidence and does not exclude the possibility that the epiblast is a second cotyledon, stronger positive evidence is still needed to prove that the epiblast in any case is a vestigial second cotyledon.

Summary

1. The embryo and the development of the seedling of the rice plant are described.

2. The general structural makeup and the provascular system of the rice embryo closely resemble that of the oat.

3. The primary root is the only seminal root present in the embryo. It is commonly a hexarch radial protostele, and lies approximately at a right angle to the axis of the epicotyl. It has three well defined histogens as commonly found among the Gramineae.

4. The root-stem transition of the vascular system takes place at the scutellar plate.

5. The scutellum is the cotyledon with a single cotyledonary (scutellar) bundle which branches only at its upper distal end.

6. The first internode contains four bundles. The relation of these

bundles with those found at the second and higher nodes is described.

7. There is a meristematic region just below the second node. The activity of the cells in this region is responsible for the elongation of the first internode during germination.

8. The coleoptile is the second leaf. Its two vascular bundles have the same relation to the cotyledonary bundle as a pair of lateral bundles of the first foliage leaf.

9. There is no vascular tissue in the epiblast, ventral scale, and coleorhiza.

10. The development of the foliage leaves is described.

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Department of Biology
National Normal University
Peiping, China

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STRUCTURAL PROBLEMS AT THE MERISTEM[1]

EDMUND W. SINNOTT

Problems of development dominate biology today. For the morphologist, the physiologist, and the geneticist it is the growing and differentiating organism, unfolding its innate capacities as it develops from fertilized egg to maturity, which occupies the center of attention. The adult plant or animal, no matter how carefully studied, cannot be understood by itself but must be interpreted by the aid of a knowledge of those stages and processes which are responsible for its final state. Such an analysis is evidently a very difficult task, and involves far more than a study of descriptive embryology alone. It is becoming the concern of almost every student of biology, and has called to its aid the biochemist and the biophysicist.

For this attack upon the problems of development, botanists are in a peculiarly favorable position. Plants are rather rigid structures and their cell walls are in general thicker than those of animal tissues. As a result, the typical mature plant cell can neither divide nor grow. Unlike the animal, therefore, the plant cannot enlarge throughout all its parts but must depend for this function on localized groups of undifferentiated and perpetually embryonic cells, the "growing points" or meristems. Meristems may briefly be defined as any regions where cell multiplication occurs. They are widely distributed in the plant body and many of them continue to function indefinitely. By their activity there arises a constant series of new organs or constant increments to old ones. Development in a plant is therefore not a single history but a continuing series of indefinitely recurring ontogenies. Its processes may be studied repeatedly in the same individual and under as wide a range of environmental conditions as the investigator can control. These advantages, together with the relatively simple character of plant

[1] Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

structure and organization, make meristematic tissues particularly favorable material for the study of developmental problems.

Despite their importance, our knowledge of the structure and activities of meristems is surprisingly meager. Early workers, following HANSTEIN's (10) promulgation of the histogen theory in 1868, endeavored to distinguish definite germ layers in the meristem. The failure of this attempt, together with a shift of botanical interest to other fields, resulted in comparative neglect of studies of the meristem for many years. More recently, however, there has been a marked revival of interest, both theoretical and practical, in such problems. No longer are we concerned primarily with the morphological equivalence of particular layers or the variation in number and shape of initial cells in different plant groups. Instead we wish to find just what determines the point of origin and the progressive development of an organ primordium; what decides whether a vegetative shoot or a flower shall be formed; what factors control tissue differentiation; and what produces differences in growth rates in the various dimensions and thus molds the form of a developing organ. The meristem is the strategic point where the fate of the plant is primarily decided, and upon this point the attention of botanists is coming more and more to be centered.

Doubtless the answers to the problem of the meristem will ultimately be given in physiological terms, but some of the most difficult ones can best be stated at present in the language of form, size, and structure. Certainly before their solution can be intelligently attempted it is essential that a detailed description, quantitatively expressed, be given for the various changes in cells and tissues which take place as the organs of the plant develop from their meristematic beginnings. The purpose of the present paper is to formulate a few of the more important of these problems in terms of structure. Their investigation is the perquisite of no one branch of biological science but requires cooperation from every field.

It should first be recognized that there are important differences in the localization and duration of meristematic activity. Some meristems are self perpetuating, whereas others are definitely limited in their duration and produce structures rather rigidly determinate as to size and shape. The former, represented in the higher plants by

the terminal meristems of root and stem and by the cambium, has received far more attention among botanists than the latter, and its activity has often been pointed out as the most distinctive feature of plant growth. One should remember, however, that only the axial parts of the plant body—the roots and stems—enlarge by means of growing points with indeterminate activity. Two important organs, the leaves and the reproductive structures, with their various parts, are definitely limited in growth and arise from diffused meristematic activity which is not self perpetuating but ultimately ceases entirely as the organ attains maturity. In the leaf this process is relatively brief, but a much longer time may elapse between the young floral primordium and completion of its growth into a mature fruit. In both the process is essentially the same. A tiny primordium, meristematic throughout, enlarges by cell multiplication and is differentiated into various parts and tissues. In some of these cell division ceases early and in others persists longer, but in all it ultimately comes to an end. After a varying amount of cell enlargement the organ then stops growing. The existence of indeterminate meristems is associated with the very loose, semi-colonial character of the plant body, which usually consists of an indefinite multiplication of similar parts or structures. Determinate meristems, on the other hand, develop in a manner much more like that of an organ of an animal, and similarly lead to the production of specific forms or patterns. Indeed the question of form determination is here presented in one of its simplest expressions, so that this type of meristem is particularly favorable for morphogenetic investigations.

Some of the fundamental problems raised by a study of the activity of both these types of meristems can be formulated most simply in terms of individual cells. Of especial significance are those concerned: (1) with cell division, (2) with cell enlargement, (3) with cell polarity, and (4) with cell shape. Each will be considered briefly in turn.

1. Mitotic cell division is the basic process underlying all meristematic activity, but it is by no means uniformly distributed. In secondary meristems it is sharply localized in a thin cambial layer. In primary meristems of root and stem it is most frequent at the tip and becomes progressively less so with increasing distance back

along the axis, until it finally ceases. In determinate meristems it is variously distributed but in some regions is more active and persists longer. Thus in certain leaves a marginal zone of dividing cells persists after mitosis has ceased in the bulk of the leaf. In the ovary of the cucurbits the entire primordium is at first meristematic, but division soon ceases in the central tissue, then progressively in the inner wall layer and outer wall layer, and finally, not long before maturity, in the epidermis. In such meristems the location and rate of cell division are evidently very important in determining growth and development.

There is also considerable evidence of a regular distribution of mitoses in time. Several students of root meristems have reported rhythmically alternating periods of high and low mitotic rate (**5**), the latter associated with more rapid cell enlargement. WAGNER (**24**) finds evidence also of a spatial rhythm at root growing points, a careful census showing two or sometimes three zones of higher mitotic rate at definite distances back from the tip.

Evidently the basic problem underlying all this is concerned with the factors which induce or prevent the process of division. The literature on this problem, both for animals and plants, is extensive and conflicting, and there is no agreement as to the paramount importance of any single factor. Cell size evidently plays a vital part, for small cells divide more rapidly than large ones, and beyond a certain size (specific for tissue or organism) division does not take place. As a cell increases in volume, its surface becomes smaller in proportion to its bulk. Mitosis has been regarded by VERWORN (**23**) and others as a mechanism for restoring an optimum relationship between these two quantities. ABELE (**1**) looks upon the surface-volume relationship of the nucleus rather than of the entire cell as determinative. HERTWIG (**11**) and others saw in the changing relation of nuclear to cytoplasmic volume the primary stimulus to mitosis. Just how all these various ratios actually effect the induction of mitosis is not clear.

Various chemical and physical factors have also been stressed as of primary importance in causing cell division. Mitogenetic radiations, long a subject of controversy, are still believed by some workers to be an important factor. Protoplasmic viscosity, as shown by

the work of KOSTOFF (**14**), FRY and PARKS (**6**), and others, is much lower during mitosis than in the resting cell; but whether this change is cause or result is not clear. The work of HAMMETT (**9**) and his school has emphasized the importance of the sulphydryl ion in stimulating division, presumably because of its role in nuclear respiration. Specific chemical substances, such as the wound hormones of HABERLANDT (**8**) and the auxins of more recent workers, seem to be definitely related to the mitotic process, at least in certain cases, although here again it is not certain whether they are inducing agents or products. Students of cellular physiology will evidently make a contribution of profound importance to all problems of development when they discover what are the ultimate factors which cause a cell to pass from the resting to the dividing state.

2. An understanding of the process of cell division alone will not solve all the problems of the meristem, however, for cells increase in size as well as in number. Students of plant growth have long recognized two rather sharply distinct phases, an early one of cell multiplication and a later one of cell enlargement only. The latter process, during which the bulk of the cell may be enormously increased, is a conspicuous and highly important aspect of growth. The extent to which the various cells enlarge determines the size of the organ and the degree of differentiation within it. This phase of growth is not in the strict sense a part of meristematic activity, however, since it occurs after cell division ceases.

There is a less conspicuous but a constant and significant increase in cell size during the period of cell division. It is remarkable how little attention this fact has received from students of the meristem. The common idea that there is a rather specific, presumably optimum, cell size for mitosis is incorrect, for in all types of meristems except cambia (and perhaps there) it seems to be the general rule that minimal cell size occurs in the youngest tissue—the terminus of the meristem or the earliest primordium—and that there is a steady increase until a rather definite size is reached, after which mitosis ceases and a much more rapid and conspicuous cell enlargement begins. This can best be seen in a simple terminal meristem like that of a root, where in passing from the apex back through the mitotic zone, an increase in cell volume amounting to several

hundred per cent often occurs. In cucurbits the tiny primordium from which an ovary develops is made up of very small cells; but as growth proceeds these cells, all meristematic, increase steadily in volume, until just before mitosis ceases they sometimes reach fifty or more times their initial volume. In a given tissue there is usually a constant relationship between the rate of this cell expansion and the rate of cell division. Where expansion is relatively rapid, the maximal cell size and the cessation of division occur early and total cell number is thus relatively small. Where expansion is slower in proportion to division, there are more divisions before mitosis ceases, and the total cell number, and consequently the ultimate size of the organ, is much greater. Similarly, where the maximal cell size for mitosis is high, there will be opportunity for more cell divisions before it is attained, and thus greater ultimate organ size, than where the maximal size is lower. If cell size is the limiting factor in cell division, the particular maximum for a given tissue or organ and the rate of expansion to this maximum are clearly very important factors in determining the ultimate size of the structure concerned.

It should be remembered that a somewhat similar phenomenon occurs even in the cambium, for SANIO (**20**)—essentially confirmed in this respect by BAILEY and TUPPER (**2**)—found that there is a continuous increase in the length of wood cells, and thus presumably of their cambial initials, for many years.

The cause of cell expansion is unknown but is obviously a part of the basic problem of growth itself. Evidently in most cases each progressive pair of daughter cells reaches a somewhat greater size than their mother cell before division again intervenes. When division ceases, this expansion, unchecked, is much more rapid and conspicuous. In its later stages enlargement seems entirely due to absorption of water. How much of it in the earlier cases results from this cause and how much from actual increase in living material is unknown, and remains to be determined by the cell physiologist.

3. But the problems of cell division and cell expansion are not the only ones which must be solved before an understanding of meristematic activity can be attained. The plane in which division occurs is also important. Mitotic figures are not random in their

orientation, but are usually more frequent in one direction or dimension than in another; indeed this is the primary cause behind the development of those specific shapes which are so characteristic of organisms. Whatever controls the direction of cell division ultimately controls organic form.

In meristems of indeterminate growth the problem is relatively simple, since division is mainly in one direction. At the very tip of the growing point of root or shoot, division is irregular, but soon most of the new walls are laid down at right angles to the axis, producing the cell rows or layers of essentially rectangular cells so characteristic of such tissue, and which convinced HANSTEIN and his followers that definite germ layers existed here. Similarly, in the cambium the great majority of the divisions are in the tangential or periclinal direction, resulting again in characteristic cell rows. Why in all these cases the cells should be more nearly four-sided than six-sided in section raises another problem, and suggests that the longitudinal walls (in primary meristems) or the radial ones (in cambia) are firmer than the walls at right angles to them.

It is in the determinate, organ-forming meristems that the problem of the plane of cell division assumes much more significance, since from these meristems structures of specific shape develop. If one makes a developmental analysis of such a structure, as of an ovary primordium, it becomes evident that cell division proceeds more rapidly in certain directions than in others. Thus in most cucurbit ovaries the wall grows much faster in length (axial dimension) than in width (radial), and this difference is found to be due to a much more rapid division rate in the former dimension than in the latter. The cells are not in rows, and the divisions are at many different angles with respect to the axis rather than merely in two; but the general average preponderance in one direction rather than in the other is so accurately maintained that the ratio between the two dimensional growth rates is constant. At the same time the internal ovarian tissue is growing more rapidly in width than in length, owing again to the more rapid division of its cells in one plane than in the other. In fruits of cucurbits there are constant differences in relative growth rates between length and width of ovary, width of placental tissue and of wall, length and width of

"neck," and width of ovary and "neck," all these differences being determined by corresponding differences in the frequency of cell divisions in various directions.

As to what determines the plane of cell division, there are again many hypotheses but no certain knowledge. The "rules" of HOFMEISTER (12) and of SACHS (19) that the wall between two daughter cells always shows a constant orientation to the axis or to the wall of the mother cell, hold good in many cases but are far from true in others. The suggestion that the position of the new wall is essentially like that which would be assumed by a weightless liquid film, proposed by ERRERA (4) and by BERTHOLD (3) and elaborated by THOMPSON (22), is physically simple and satisfying, and is undoubtedly applicable in certain cases; but there are many others which it cannot explain and it clearly leaves out of consideration a number of important biological variables.

The problem of plane of cell division evidently involves primarily the polarity of the cell, or more probably, as GIESENHAGEN (7) suggests, the polarity of the nucleus. Whatever it is that orients the mitotic figure must be the chief factor in determining where the new cell wall is to be laid down, and thus the direction of growth of the two daughter cells. Various factors have been suggested as determinative. The first division in isolated cells is usually at right angles to gravity. It has been shown by KNY (13) and others to be parallel to the direction of applied pressure, and by STAHL (21) and others to be at right angles to incident light. More recently LUND (16) has stressed the importance of electric currents in determining polarity. WHITAKER (25, 26) and his students (15) have shown that the position of neighboring cells, the visible cell constituents as rearranged by centrifuging, and gradients in temperature and in hydrogen ion concentration, all are related to the induction of polarity; and OLSON and DU BUY (18) have found auxin similarly effective. Most of these investigations have been carried on with single cells, notably the eggs of *Fucus* and spores of various sorts. What seems especially needed is similar work with masses of meristematic cells, toward which the studies of NEEFF (17) on the experimental alteration of the polarity of cambial cells point the way. When the cell physiologist can determine precisely what are the ultimate factors

which control cell polarity, and how they operate, he will have made a significant contribution to our understanding of development. It is at this point that physiology and morphology come most closely into contact, and here seem to focus some of the ultimate problems of biological science.

4. The fourth problem is that of change of form among meristematic cells. Most of these are isodiametric, and the bulk of change in cell shape occurs during the process of differentiation after division has ceased. Even in the meristematic tissues, however, there are the beginnings of considerable cellular differentiation. Thus the elements of the provascular strands consist, even while division among them is still going on, of cells markedly elongated parallel to the axis of the organ. In relatively small ovary primordia of cucurbits the inner layer of the wall consists of cells elongated parallel to the axis, and the outer layer of cells tangentially elongated. Both types become isodiametric later but are far from being so in their early stages. In all such cases the suggestion is obvious that cell polarity is also involved. The role of plant hormones in determining cell elongation through their effect, direct or indirect, on wall growth has been studied by many recent workers and provides the only definite suggestion at present for the origin of differences in cell shape.

These four major structural problems in the meristem: cell division, cell enlargement, cell polarity, and cell shape, have all been approached here from the point of view of the cell rather than of the cell aggregate. There remain the more complex matters, such as the origin of leaf primordia through the more rapid growth of surface layers in comparison with those beneath, the change in form of the meristem during the course of a single plastochrone, or the differences in the shape of the meristematic cone which are associated with the production of primordia of vegetative and of reproductive organs respectively. With these should also be considered the important physiological studies which have concerned themselves with meristematic changes. The effects of mineral nutrients, of differences in light intensity and in photoperiod, of carbohydrate-nitrogen relation and of growth-differentiation balance, are among the more important of these researches. Nevertheless beneath all

these more complex developmental phenomena lie those changes in individual cells to which attention has here been directed. There well may be integrative factors which control mass phenomena in development, but these must operate through changes in number, size, position, and form of individual cells. To describe precisely what happens at the meristem, in terms of the structure of cells and cell aggregates, is the important task of the cytologist and the morphologist. An ultimate solution of the problems thus formulated must depend upon many biologists, but especially upon students of cellular physiology. When through such cooperation we gain a clearer understanding of the processes which go on in these determinative regions, we shall be well on the way toward a solution of the major problems of plant growth and development.

BARNARD COLLEGE
COLUMBIA UNIVERSITY
NEW YORK

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RELATION OF CONDENSATION REACTIONS TO MERISTEMATIC DEVELOPMENT[1]

W. E. LOOMIS

Introduction

Evidence from many sources indicates that vegetative apical meristems are commonly dominant in correlative plant development. Examination of an active apical meristem of a shoot or root axis shows a mass of dividing cells at or near the tip of the axis. Cells within an easily recognizable apical zone continue to divide as long as temperature, moisture, food, and related factors are favorable. Cells left behind by the advancing tip either cease division entirely or divide slowly and irregularly during the period of enlargement. The cells in the region of division are characterized by a dense, relatively unhydrated protoplasm and by the rapid rate at which they are able to condense amino acids and similar nitrogenous compounds into new protoplasm.

Meristems such as the vascular cambium, root initials during early stages of their formation, growing flowers, intercalary meristems in grasses, etc., show no comparable massing of dividing cells, and these diffuse meristems are characteristically less effective than the massed meristems in utilizing and in competing for the materials of which the cell is constructed. We suggest as a working hypothesis for explaining these reactions, that masses of meristematic cells are able to build up conditions favorable to protoplasmic condensations while similar reactions can occur in diffuse meristems only under more favorable circumstances, or with the assistance of factors external to the meristem.

Condensation and cambial development in Populus

A number of workers have shown that cambial growth in deciduous trees is initiated subsequent to shoot growth, and that it starts

[1] Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

at the tip of the shoot. It has been shown previously (**6**) that cambial development in apple (*Pyrus malus* L.), poplar (*Populus nigra* L.), and boxelder (*Acer negundo* L.) was promptly arrested at any time during the growing season by removing a ring of phloem above the test region. If leaf-bearing branches were present below the ring, cambial growth continued below the branches. If the ring was made below the lowest branch, all cambial development of the shoot below the ring ceased quickly, while secondary growth above the ring proceeded for a time at a normal or accelerated rate. Chemical analyses of xylem and phloem showed that cambial division was correlated with a relatively low concentration of soluble organic nitrogenous compounds; and shoot initiation, such as occurred below the rings, with a relatively high concentration of these substances. With the three species used, one showed high mono-amino nitrogen correlated with development of massed (shoot or callus) meristems, the second showed high basic nitrogen, and the third a residual fraction neither mono-amino nor basic. Apparently any of a number of organic nitrogenous compounds may be condensed into protoplasm when conditions are favorable.

More recently AVERY, BURKHOLDER, and CREIGHTON (**1**) have shown that initiation of cambial growth in woody twigs is preceded by formation of growth promoting substances within the buds and its polar export down the twig. Growth substances, however, could not be isolated by diffusion from the phloem of the trunk or larger branches of trees showing rapid cambial development. They failed also to obtain a correlation between the quantity of growth substances diffused from the terminal buds and the rate of cambial development. By July 16 the growth hormone production had dropped to less than 5 per cent of its May peak, yet cambial division is commonly proceeding at a maximum rate during July and August.

In an earlier paper (**6**) the writer questioned the role of hormones in cambial growth because of the apparently dominant effect of mature leaves. A number of recent experiments, the details of which will be published elsewhere, have shown beyond question the possibility of stimulating cambial development in *Populus* with growth substances. Microscopic measurements have shown cambial stimu-

lation by etiolated and by defoliated shoots, as well as by normal shoots in the light, and direct stimulation of cambium, both in one-year-old twigs and in eight-year-old trunks of *Populus balsamifera* L., by a 0.2 per cent paste of heteroauxin in lanolin. Chemical studies of the regions in which cambial growth has been stimulated have shown a statistically significant reduction of the organic nitrogenous compounds soluble in 80 per cent alcohol (7) in all but one experiment. In this experiment, with small disbudded plants treated with heteroauxin, rapid upward translocation in the absence of shoot growth appears to have offset the expected decrease in soluble nitrogen. Data on growth and soluble organic nitrogen from several experiments are included in table 1. Cambial growth was varied by: (a) Cutting away the phloem above or below the test segments. (b) Position above or below a side branch on a trunk segment isolated by phloem rings; cambium developed only below the side branch in such a segment. (c) Growing in darkness and in light, with and without disbudding; etiolated shoots stimulated cambial growth but at a reduced rate. (d) Applying heteroauxin pastes. All treatments which increased cambial growth decreased the percentages of soluble organic nitrogen. Changes in total organic nitrogen showed inconsistencies associated with storage and digestion of materials not directly related to growth. The trend was toward increased insoluble nitrogen in the regions showing cambial development.

The data may be explained by assuming that the diffuse meristem, the cambium, was unable to condense the soluble organic nitrogenous compounds formed by digestion of stored proteins. With the aid of leaves in the light, growing etiolated buds (supplying auxin?), or external supplies of heteroauxin, conditions were made more favorable for condensation reactions, and the cambial cells were able to synthesize protoplasm and to divide. A causal relationship between condensation and cambium development is indicated by the suddenness with which cambial division ceases and soluble organic nitrogen concentrations rise below phloem rings cut during periods of rapid cambial growth.

It has been suggested (6) that the direct condensation of nitrogenous compounds in lighted, mature leaves may explain their effect upon cambial development. A condensing effect of auxins and the possibility of non-diffusible forms of auxins being produced in

mature leaves and moved through the phloem must be added to this concept, but direct condensation and translocation of partially condensed protein forms from the leaves may still be one of the reactions concerned. Such condensation would explain midsummer cambial growth when hormones are low or nondetectable by diffusion methods, and the effect of the removal of mature leaves, shown by

TABLE 1

CAMBIAL GROWTH AND SOLUBLE ORGANIC NITROGEN IN POPULUS BALSAMIFERA

Sample	Age (years)	Cambial growth		Soluble nitrogen	
		Microns	Difference	Milligrams per 100 grams	Difference
Trunk below ring	8	142		110	
Trunk above ring	8	1036	+894*	67	−43*
Trunk below ring	8	141		99	
Same plus heteroauxin	8	302	+161*	84	−15†
Below ring, above branch	8	124		113	
Same but below branch	8	353	+229*	80	−33*
Between rings	8	169		113	
Same plus heteroauxin	8	245	+ 76*	99	−14*
Check in dark	1	44		187	
Check in light	1	135	+ 91*	143	−44*
Buds removed	1	0		228	
Same plus heteroauxin	1	54	+ 54*	219	− 9

* Highly significant; below 1 per cent level.

† Significant; probability of difference being due to sampling is less than 5 per cent.

PROEBSTING (11) to stop cambial growth in apple. In one of our experiments the soluble organic nitrogen of etiolated poplar leaves was reduced from 0.486 to 0.087 per cent by exposing them to normal greenhouse light for a week. Total nitrogen of the leaves decreased so that the condensed materials were apparently exported from the leaves. A sharp, although less marked, drop in the soluble nitrogen of the bark was evidence that the surplus nitrogen was not exported in unchanged form. Many other experiments could be cited which indicate that nitrogen condensation occurs in the light in mature leaves where auxin formation appears to be low or absent.

Condensation and root formation in Melilotus alba

Dr. J. N. MARTIN of the Department of Botany at Iowa State College has shown in unpublished data that initiation of new branch roots in sweet clover, *Melilotus alba* Desr., is dependent upon the presence of shoots growing in the light. Roots planted in the greenhouse late in the fall of their first year, and decapitated to remove the cotyledonary buds, formed a few unbranched secondary roots, apparently from preformed root initials. If these first root tips were removed no further growth occurred unless a shoot was grafted onto the root. Reserves of carbohydrate and nitrogenous materials in the treated roots were high, and the massed meristems of preformed root

TABLE 2

SECONDARY ROOTING AND SOLUBLE ORGANIC NITROGEN IN MELILOTUS ALBA

TREATMENT	AVERAGE DRY WEIGHT ROOTLETS (MG.)	PERCENTAGE		
		DRY MATERIAL IN ROOTLETS	SUGARS IN ROOTS	SOLUBLE NITROGEN IN ROOTS
Control, as planted	.....	.....	11.59	0.58
Planted, decapitated	41	9.5	5.22	0.57
Normal, grown in dark	93	6.6	1.67	0.45
Normal, grown in light	290	8.2	1.82	0.40

tips grew at a normal or greater than normal rate. The difficulty apparently arose from the inability of the decapitated roots to form new root initials in the diffuse meristems of cortex or pericycle. Growth data from one of MARTIN'S experiments with chemical analyses by the writer are shown in table 2.

Organic reserves were higher in the planted roots than the sugar percentages indicate, since there was a shift from sugar to starch in the roots held at greenhouse temperatures. Heavy shoot growth, covered to exclude light, had some stimulating effect on root growth in spite of the more rapid reduction of organic reserves. The low percentage of dry matter in the rootlets of these plants was probably the result of utilization by the tops of sugar which would otherwise have been used in differentiation of the roots. The reduction in the soluble organic nitrogen of the sprouting roots might have been assigned to utilization by the tops were it not that soluble nitrogen

percentages tend to be independent of utilization until protein reserves are depleted, a condition which was not reached in this experiment.

The striking increase in weight of rootlets on plants with their tops exposed to normal December greenhouse light was the result of the formation of more secondary roots and of profuse branching of all rootlets. In contrast, the rootlets of the darkened plants branched sparsely and those of the decapitated plants not at all. Light on leaves appears in this experiment to have been the important factor in root development. The results with etiolated plants were intermediate and resemble the results obtained with cambial growth in etiolated poplar. Soluble organic nitrogen was inversely correlated with root formation, and the explanation suggested for poplar appears to be applicable here. An increased tendency toward nitrogen condensation was accompanied by activation of a diffuse meristem, in this instance the pericycle.

Condensation of nitrogenous compounds and intercalary growth in first internode of maize

VAN OVERBEEK (12) has shown that height growth in maize, resulting almost entirely from intercalary growth, is dependent upon auxins produced by the staminate inflorescence. When the normal auxin supply is inhibited or destroyed, a dwarf type of corn is produced. INGE and LOOMIS (4) have found that intercalary growth in the first internode of germinating maize is dependent upon an uninterrupted supply of auxin from the coleoptile tip. If the coleoptile tips were irradiated, heated to 50° C., or decapitated several times at intervals of a few hours, intercalary division ceased, the embryonic region became differentiated, and the first internode lost its capacity for further growth. If the natural supply of auxin, cut off by treatment, was replaced by applying heteroauxin pastes, the treatments were ineffective in preventing growth. The massed meristems of the plumule and nodal roots were inhibited by auxins and started rapid development only after internodal growth had been arrested. The same balance between diffuse and massed meristems found in poplar appears to be involved in this correlation. Chemical analyses of plants showing internodal (diffuse meristem) growth and of

plants in which internodal growth had been stopped and plumule (massed meristem) growth stimulated, are included in table 3.

The control plants were grown in complete darkness. The lighted plants were germinated in darkness for three days and were then exposed for twenty-four hours to Mazda light at 100 f.c. This treatment is approximately the minimum illumination required to produce prompt and complete suppression of internodal growth in maize. The second lot of plants was handled in the same way except

TABLE 3

CHANGES IN SOLUBLE NITROGEN OF MAIZE SEEDLINGS ILLUMINATED FOR 24 HOURS WITH MAZDA LAMPS AT 100 F.C.

TISSUE	MILLIGRAMS SOLUBLE N IN 100 GM.			
	CONTROL	LIGHTED	DIFFERENCE	"t"
After 24 hours light				
Internodes	154	200	+46	8.1*
Plumules and coleoptiles	128	152	+24	6.5*
48 hours later				
Internodes	166	209	+43	9.0*
Plumules and coleoptiles	135	145	+10	4.0†

* Highly significant (1 per cent level).
† Significant (5 per cent level).

that the lighted plants were returned to the dark chamber and held, with the controls, for two days after being illuminated. The treated plants showed rapid plumule and nodal root growth during these two days but made no further internodal growth. In both experiments the plumule leaves did not push through the coleoptiles of the controls, and internodal growth was proceeding normally at the time the chemical samples were taken. The internode samples consisted of the entire first internode, while the plumule samples included the coleoptile and first node.

In the experiments with poplar, illumination reduced the soluble nitrogen in both the leaves and the phloem. Maize presents the apparently anomalous effect of an increase in soluble nitrogen, an increase which persisted after the plants were returned to darkness. The results obtained are considered to be due to the dominating

role of auxins in the maize correlation. The coleoptile tips of germinating grasses have been shown by many workers (**3, 14**) to produce or to activate growth hormones in relatively large quantities. These auxins are exported polarly downward, and are considered to be the external factor which stimulates cell division in the diffuse, intercalary meristem of the first internode. As in the previous experiments, cell division in a diffuse meristem occurred in a tissue characterized by a high degree of polymerization of organic nitrogen compounds.

When the supply of auxins from the coleoptile tips was reduced by illumination there was a rapid increase in the less condensed forms of nitrogen, and growth shifted from the diffuse to the massed meristems of the plants. The responses in three diverse types of meristematic correlations are so uniform that causal relationships appear probable.

Discussion

The data here presented can be explained by assuming that the condensing power of diffuse meristematic tissues is low, and that rapid cell division in such tissues is possible only when their ability to condense simple organic nitrogenous compounds into protoplasm is augmented by external factors. One of these factors appears to be a continued supply of auxins. The action of natural or artificial supplies of growth substances has been demonstrated for all three of the correlations studied: cambium activity (**1**), root initiation (**13**), and intercalary growth (**4, 12**). Although not so well demonstrated, the possibility of the activation of diffuse meristems by direct supplies of condensed proteinaceous materials from lighted leaves must be considered. The present data show that condensation of simpler organic compounds occurs rapidly in lighted leaves. The data of MASKELL and MASON (**8**) indicate that proteins, as such, are exported from the leaves of cotton. On the other hand, there is much evidence that stored proteins are re-translocated only after digestion to simpler forms. It appears necessary to postulate further condensation or denaturization reactions during storage which reduces the mobility of the proteins.

During the spring, or whenever the leaf area of the plant is suddenly reduced or separated from the storage tissue by a phloem ring,

stored (or protoplasmic) proteins are digested to simple forms and sprout growth is stimulated. In this connection it is important to recall that sprout growth is stimulated also by the addition of nitrogenous fertilizers (**5, 10**) which result in an increase in the soluble organic nitrogen of the plant (**5, 9**). The production of auxins is probably increased by the added nitrogen and greater sprout growth (**2**), but normal balance of growth is not restored until the leaf area has been increased. During the summer, when cambial activity is greatest, the auxin level of the plant drops to a low value (**1**), but a balance of condensation favorable to diffuse meristems continues unless the plant is defoliated (**11**) or ringed above the region of cambial growth (**6**), in which cases cessation of cambial growth and change in nitrogen balance may be observable within twenty-four hours. All of these reactions suggest that diffuse meristems (the cambium) are stimulated by, and are able to utilize directly, intermediate forms of protein condensate supplied by the leaves. The intercalary growth of maize must be considered to be an exception to this generalization, for height growth in dwarf lines is not stimulated by light (**12**).

The effect of auxins upon development of diffuse meristems offers many interesting problems. Does auxin act directly in condensing nitrogenous compounds? Does it stimulate action in the meristematic cells themselves or does the condensation occur within the phloem? Is there any relation between this action of auxin and the inhibiting effect which it has on massed meristems?

Heteroauxin pastes inhibit bud development in poplar and decrease the concentration of the simpler nitrogenous compounds in the phloem. These pastes also inhibit development of marginal buds in *Kalanchoe*, but without inducing detectable changes in the nitrogenous compounds of the leaf blade tissue. These results suggest that the reaction occurs within the phloem which is inactive in detached leaves of *Kalanchoe* and which constitutes such a small percentage of the leaf that condensation which may occur in this tissue is not determinable by analysis of the entire blade. This hypothesis of condensation within the phloem is supported by the data for maize (table 3). The internodes taken for the chemical samples in the controls were 10 to 15 cm. long. The intercalary meristem occupied scarcely more than 1 per cent of this length, yet marked differences

due to light treatment were observed when the entire internode was analyzed twenty-four hours after initiating the treatment. Phloem action would be augmented in this tissue, of course, in comparison with the leaf of *Kalanchoe*, by the fact that large quantities of proteinaceous materials are being moved upward from the seed, so that actions occurring within the phloem would assume an importance entirely disproportionate to the mass of this tissue.

Summary

1. Cambial growth in *Populus balsamifera* L. has been stimulated by (a) etiolated buds, (b) leaves in the light, and (c) applied heteroauxin pastes. In all the experiments, shoots showing cambial activity were characterized by a reduced percentage of soluble organic nitrogen in the phloem and wood of the dividing region.

2. Initiation of new root tips in *Melilotus alba* Desr. did not occur in decapitated plants brought into the greenhouse in November. Branching was increased slightly by the presence of etiolated buds, and markedly by the presence of leaves in the light in either normal or CO_2 free air. Increased root formation was accompanied by decreased percentage of soluble organic nitrogen in the storage roots.

3. Elongation of the first internode of *Zea mays* L. continued for a week or longer but was stopped by illuminating the tips of the plants for twenty-four hours with a Mazda lamp at a light intensity of 100 f.c. The plants showing intercalary growth were characterized by low percentages of soluble organic nitrogen.

4. In the three species studied, diffuse meristems, cambium of poplar, pericycle of sweet clover, and intercalary meristem of maize were active when the soluble organic nitrogen content of the tissue was relatively low and inactive when it was relatively high. At high percentages of soluble organic nitrogen the massed meristems of stem and root tip were stimulated to begin or increase their development.

5. A hypothesis of condensing power is set up to explain the observed facts. According to this hypothesis the massed meristems are able to utilize simple organic forms of nitrogen and to condense these forms into protoplasm in the formation of new cells. The diffuse meristems are less effective or ineffective in condensing simple nitrogenous forms, and can develop only when supplied with

partially condensed forms of protoplasm building compounds, probably with simple albuminoid proteins.

6. The condensed forms required by diffuse meristems may be synthesized in darkness in the presence of auxins, with the phloem indicated as the seat of the reactions; or they may be formed in lighted leaves, possibly independently of known growth substances.

IOWA STATE COLLEGE
AMES, IOWA

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INFLUENCE OF PHOTOPERIODS UPON THE DIFFERENTIATION OF MERISTEMS AND THE BLOSSOMING OF BILOXI SOY BEANS[1]

H. A. BORTHWICK[2] AND M. W. PARKER[3]

(WITH EIGHT FIGURES)

Introduction

Photoperiodism, discovered by GARNER and ALLARD (2) in 1920, has attracted the attention of many investigators who have attempted to find a physiological explanation of the results observed. GARNER (1) and MURNEEK (3) in recent papers give rather complete literature summaries. MURNEEK and GOMEZ (4) and MURNEEK (3) recognize that while many data have been accumulated descriptive of the physiological processes accompanying change of photoperiod, the causes of the reaction are still unknown. It is probable that many of the physiological studies of photoperiodism have dealt with plants in which the change in the meristems in response to photoperiod was so far advanced that the physiological conditions involved in the differentiation of flower parts were not recognized. The conditions associated with change in form of the plant may have become obscured by changes correlated with development subsequent to the earliest stages of floral initiation. It is desirable to study the physiological condition before and during the time when the shift in the type of derivative from the meristem is from strictly vegetative to floral.

Outline of procedure

In this work a study of the occurrence and rate of development of flower primordia of the Biloxi soy bean has been made before and after the plants were subjected to various photoperiods. The objects of the experiment were: first, to determine the minimum number of short photoperiods necessary for initiation of flower buds;

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[2] Morphologist. [3] Associate Physiologist.

second, to compare photoperiods of various lengths as to their effectiveness in causing initiation and later development; and third, to determine certain after-effects of treatment that could not be measured until late in the development of the plants. Young seedlings were grown for about a month on a photoperiod long enough to keep them vegetative. They were then subjected for various numbers of days to a wide variety of photoperiods, many of which were short enough to cause initiation and development of flower buds. Following these treatments, the longest of which extended over a period of 10 days, the plants were placed on the natural photoperiod of about 15 hours then prevailing to continue their development. As the season progressed, the natural photoperiod gradually decreased in length to approximately 11.5 hours at the conclusion of the experiment.

About 1500 Biloxi soy bean plants were started May 28 and were grown for 40 days (until July 6) on a day length of about 17 hours. This length of photoperiod was obtained by extending the natural day from sundown until 10 P.M. by means of Mazda light. On July 6, when differential treatments were started, the plants were about 1 foot high and had five expanded compound leaves. A careful microscopic examination of all growing points on thirty-two of these plants showed that no flower primordia were present.

On this same date 1252 of these plants were selected for the experiment. One hundred were reserved as controls and were transferred directly from a 17-hour photoperiod to the natural photoperiod of about 15 hours then prevailing. The remaining 1152 plants were divided into eight series of 144 plants each. Each series was subjected to a different schedule of photoperiodic treatments (table 1).

After one day of treatment a lot of eighteen plants from each series was placed on natural photoperiod. On the second day another lot of eighteen plants was removed from each series, and so on until all the plants were used. There were eight such transfers, made at the end of 1, 2, 3, 4, 5, 6, 8, and 10 days. Thus in all there were sixty-four different experimental lots and a group of control plants.

The photoperiods for these experiments were so arranged that the midpoints of each occurred at twelve o'clock noon. This was done

so that the plants on very short photoperiods might be exposed to high light intensity. Thus the plants in any given series received on the last day of treatment a longer photoperiod than that scheduled. This final photoperiod was determined by the number of hours of daylight to which the plants were exposed after being removed from the dark chambers. The length of this final photoperiod ranged from 9 hours for the plants kept in darkness to 15 hours for the plants scheduled for a 14-hour photoperiod, as shown in table 1.

TABLE 1

SCHEDULE OF PHOTOPERIODS OF VARIOUS SERIES OF TREATMENTS

	Series							
	A	B	C	D	E	F	G	H
Length of photoperiod on all days except last of each treatment, in hours	0	2	4	6	8	10	12	14
Length of photoperiod on last day of treatment, in hours	9	9	10	11	12	13	14	15

As already mentioned, the plants at the beginning of the experiment were known to have no flower primordia. Microscopic examination of control plants from day to day under natural day conditions showed that the flower primordia were first evident the last week in July. Since the scheduled differential treatments were completed on July 16, any flower primordia present at that time must have been produced in response to the treatments imposed.

Observations

Flower bud initiation

The experimental plants of the various series were examined daily for the presence of flower primordia. On the fifth day these were evident in certain lots, and between this time and the tenth day, plants from all experimental lots were examined. These observations (fig. 1) show that the stimulation brought about by two short photoperiods was sufficient to alter the course of development of the

meristems in such a way that differentiation of flower primordia resulted. Flower primordia were not visible at the end of two days of treatment, but three days later, during which time they were on natural day length, which was then greater than the critical, the

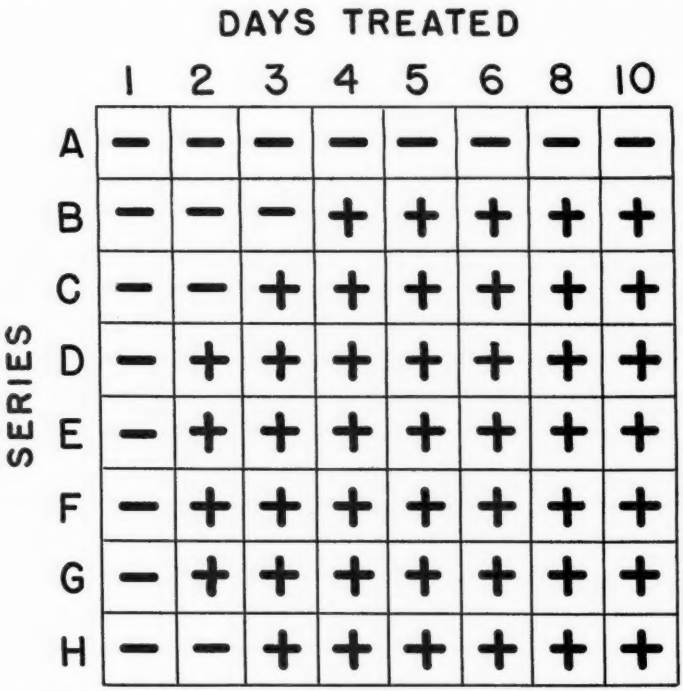


FIG. 1.—Effect of various numbers and lengths of photoperiods upon differentiation of flower primordia. Final observations made tenth day after start of differential light treatments.

beginnings of flower primordia were evident and at the end of ten days they were very conspicuous. Since two short photoperiods were sufficient to set in motion the mechanism that makes possible the differentiation of flower buds, it is obvious that fundamental physiological changes occurred during that brief period.

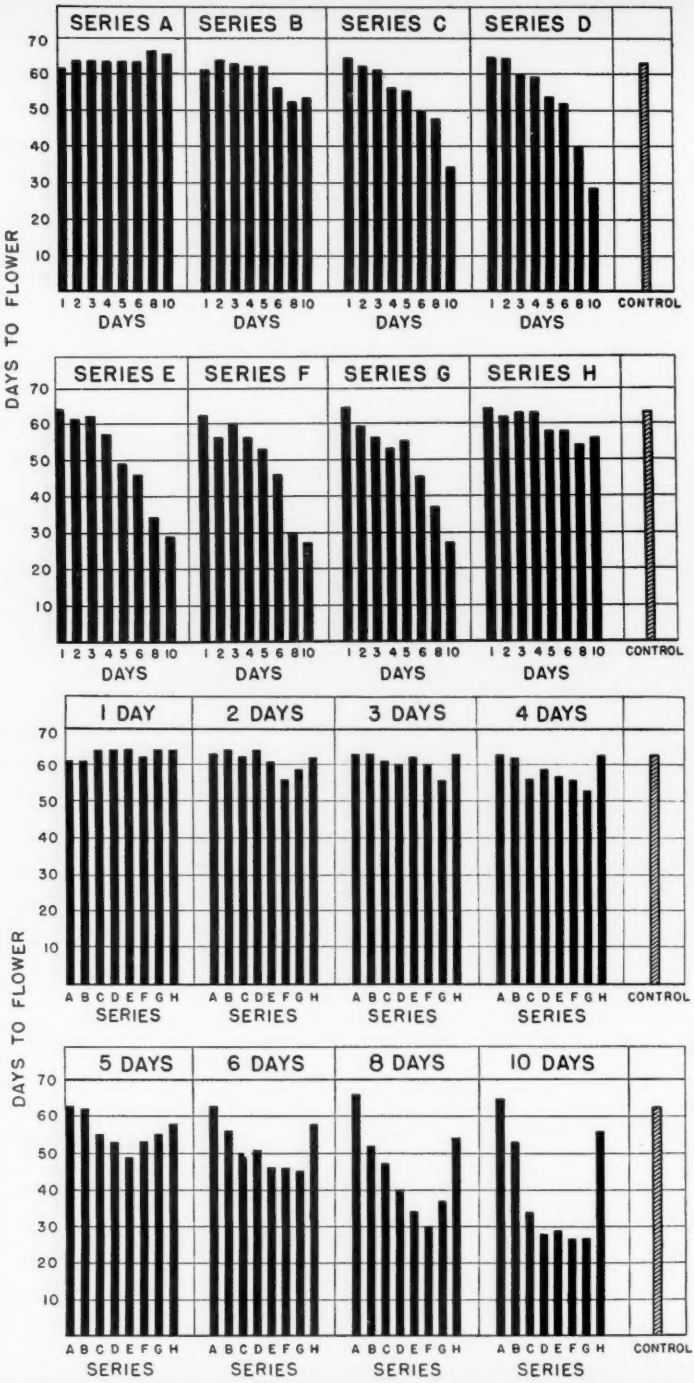
Time of flowering

The date of opening of the first flowers on each of eight plants in every treatment and in the controls was recorded (figs. 2, 3). It was found that all of the lots subjected to complete darkness for periods of 1 to 10 days and then placed under the conditions of natural day blossomed at about the same time as the controls (series A, fig. 2). This result would have been expected from the data shown in figure 1, for it will be recalled that no flower primordia had been differentiated during the time of treatment. The photoperiodic stimulation that finally caused them to blossom was evidently received later in the season, after they were returned to natural day. In the lots of series H (table 1), on the contrary, flower primordia were differentiated during the period of treatment but these plants also blossomed nearly as late as the controls. The plants in series D, E, F, and G (table 1), exposed only twice to their respective photoperiods, initiated flower buds, but additional days of treatment progressively hastened the opening of flowers. The first stages of flower bud initiation occur as the result of a few photoperiods that range in length from 2 to 14 hours. Subsequent development of these flower buds and their time of blossoming, however, appear to be greatly influenced by the length of the photoperiods and their number.

Structure of growing points on long day

It is evident that the structure and development of the plant, particularly its growing points, must be well known in order that the first indications of flower primordia may be recognized.

The soy bean plant growing on a photoperiod of sufficient length so that it remains vegetative develops as follows: Since the leaf primordia at the tip of the main axis and in the lateral buds are 2-ranked, a median longitudinal section cut in one plane passes through successive pairs of overlapping stipules (fig. 4*a*), and cut at right angles to this plane, passes through the midribs of the leaves and any axillary buds present (fig. 4*b*). The lateral buds as seen in figure 4*b* do not appear to develop any leaf primordia until they attain considerable size. Actually, however, leaf primordia are pres-



FIGS. 2, 3.—Effect of different numbers of photoperiods of various lengths upon time of opening of first flowers. Days to flower expressed as number of days after beginning of differential light treatments, July 6. Each bar represents mean value obtained from eight plants.

ent in the bud in the axil of the fifth leaf from the tip. To demonstrate this, longitudinal sections of the bud must be made in a plane across the leaf axil (fig. 5). The primordium in the axil of the fourth leaf from the tip of the plant as seen in this plane consists of a ridge elongated across the leaf axil (fig. 5*a*). In the next older bud a leaf primordium is seen at each end of this ridge (fig. 5*b*). These are the

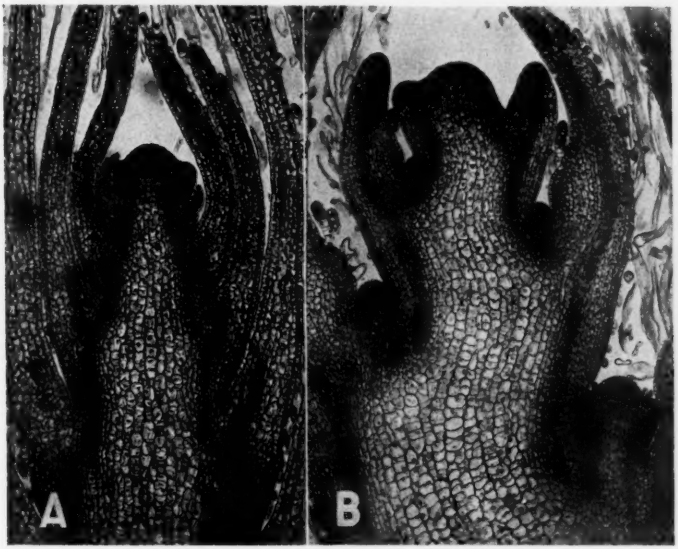


FIG. 4.—Longitudinal sections through growing point of main stem of plants grown on long day: *A*, plane of section passing through successive pairs of overlapping stipules; *B*, same through midribs of leaves and axillary buds.

two prophylls which are present at the base of every vegetative branch. In the axil of each of these a bud is ultimately differentiated. The next older bud contains these primordia and also the primordium of the first compound leaf (fig. 5*c*). After the growing points of the side branches reach this stage of development they repeat the behavior of the terminal of the main stem. Each successively older bud is like the one immediately above but contains one additional compound leaf. This seems to hold true until six or eight compound leaves are formed in the bud, after which the growing point becomes apparently less active. Although there may be oc-

casional deviations from this pattern, there is remarkable uniformity so long as a photoperiod of 16 or more hours is maintained.

Development of growing points under short photoperiod

If the photoperiod is shortened to 14 hours or less there is a marked change in the early stages of bud differentiation. This change might easily escape notice in sections not cut in the proper plane or in dissected fresh material, unless all growing points are critically

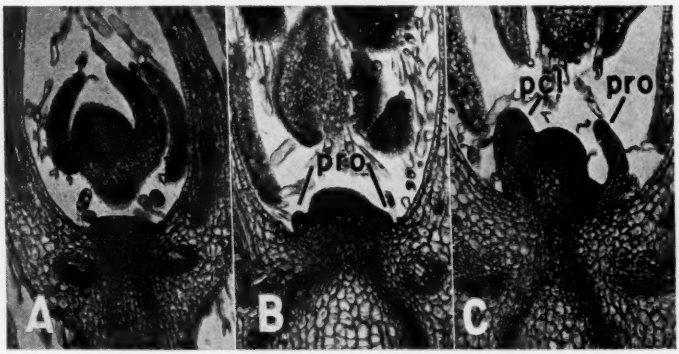


Fig. 5.—Longitudinal sections of young axillary buds of plant grown on 16-hour photoperiod. Buds *A*, *B*, and *C* located in axils of 4th, 5th, and 6th leaf primordia respectively from apex of main stem. *pro*, prophyll primordia; *pcl*, primordium of compound leaf.

examined. For example, in the axil of the fourth or fifth primordium from the tip the bud appears as in figure 6*a*, and as it grows older it goes through the stages shown in figure 6*b* and 6*c*. There are two prophyll primordia differentiated as usual, but there is almost immediately active development of the buds in their axils. The primordia of the prophylls do not seem to develop so rapidly on the flowering branches as they do on strictly vegetative ones, but the buds in their axils are relatively much more active.

The next leaf primordium above the prophylls on a flowering branch forms a small bracteal leaf, whereas it forms a large compound leaf on a vegetative branch. The difference between these structures is evident as soon as the primordia can be distinguished under the microscope, because the latter has stipules and the former

has not. In the axil of this bract there is also very early development of a bud. Usually there is a rapid succession of these bractlike primordia, the number apparently being influenced by the photoperiod and possibly other environmental factors. Instead of being 2-ranked, these primordia are spirally arranged. Each of the buds that arises in their axils gives rise directly to a single flower. In the development of the flowers a similar pair of prophylls is produced. In the flower primordium the lower part of the branch ma-

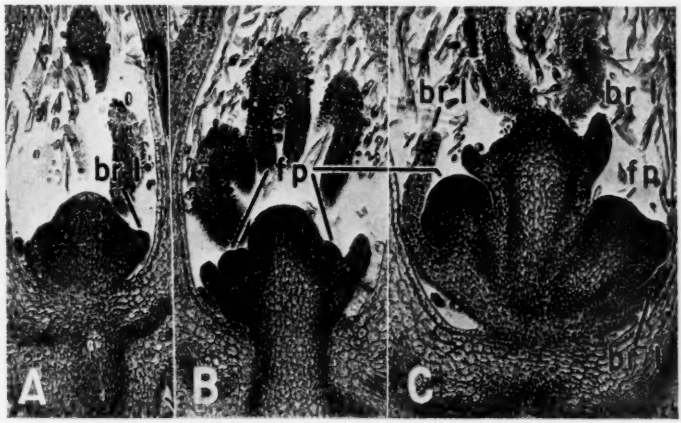


FIG. 6.—Longitudinal sections of young axillary buds of plant transferred from long to short photoperiod about 10 days before material was collected. *fp*, flower primordium; *br l*, primordium of bracteal leaf.

tures into the pedicel, however, and in its elongation the prophylls are carried outward and appear as a pair of bractlike structures, inserted on each side at the base of the receptacle. After forming the primordia of these bracts, the growing point gives rise directly to the various whorls of flower parts.

LOCALIZATION OF FIRST VISIBLE RESPONSE TO SHORT DAY

The first morphological change induced by short photoperiod is very definitely localized. The main axes of thirty-two plants dissected on the day the photoperiodic treatments were started averaged seventeen nodes, with relatively little variability from that mean (fig. 7). Although the axillary buds of such plants are in-

conspicuous, they actually contain the primordia of many vegetative leaves, some of which have buds in their axils.

When a plant of this type is subjected to five or six short photoperiods, the first evidence of morphological change is found in the

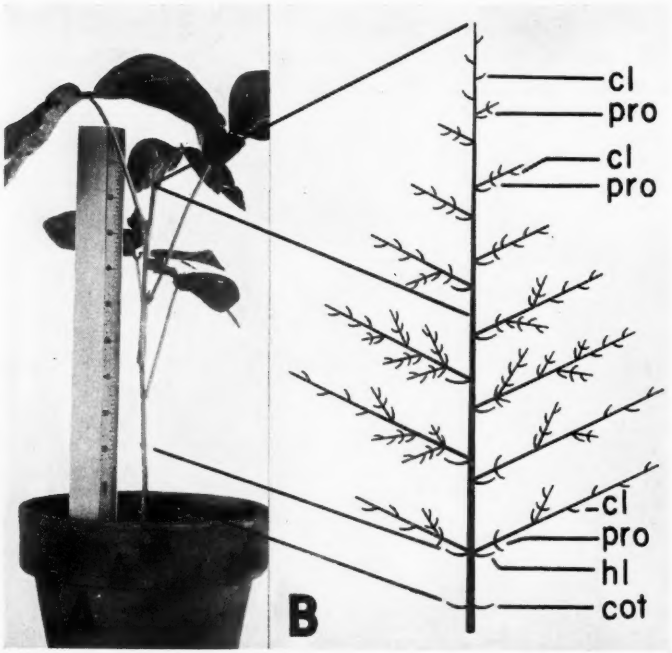


FIG. 7.—*A*, plant of approximately the same size as those used in these experiments, grown on 16-hour photoperiod. Five expanded compound leaves and total of seventeen nodes in main axis. *B*, diagram showing number and position of various structures present on plant shown in *A*. *pro*, prophyll; *cl*, compound leaf; *hl*, heart shaped leaves; *cot*, cotyledon.

bud located in the axil of the leaf primordium fourth or fifth from the tip of the main stem. Similar conditions prevail in some of the axillary buds, whereas in others there is no immediate response. This behavior of the axillary buds may be correlated with their physiological condition.

The first evidence of flower bud formation on the experimental

plants was usually found in the axil of the leaf at node fifteen, and was identified as such approximately a week after the beginning of treatment with shortened photoperiods. This bud subsequently developed into a small inflorescence consisting of a short branch bearing two reduced prophylls and two bracteal leaves, each of the latter with a single flower in its axil. The tip of the axis differentiated no additional leaves.

If the plant had not been subjected to short photoperiods this axis would have differentiated its two prophylls as usual, but the additional leaves formed would have been compound instead of bracteal. The branch would have elongated and the growing point would have continued to differentiate vegetative leaves.

At the time the differential light treatments were started, no external evidence of differentiation of a bud was apparent in the axil of the leaf primordium at node 15. In longitudinal sections through the region, however, the first stages in differentiation of the axillary growing point were evident (fig. 8). Such buds as show the earliest response to short photoperiods are in an exceedingly early stage of differentiation when the treatment is begun, and their growing points never give rise to primordia of compound leaves but produce bracteal leaves directly after the prophylls are differentiated.

After-effects of treatment

Those growing points which are fully organized and producing primordia of compound leaves at the start of the photoperiodic treatment behave differently. The main stems of flowering soy bean plants are eventually terminated by inflorescences. In these inflorescences the flowers occur singly in the axils of bracteal leaves borne on the main stem. From this it is apparent that the growing points which during the early stages of development of the plant give rise to the primordia of compound vegetative leaves eventually give rise to primordia of several bracteal ones before they cease functioning.

At the beginning of the experiment, the plants had an average of seventeen nodes with compound leaves or their primordia located at the fifteen uppermost. The number of compound leaves present on the main axis at the end of the growing season was determined, and from this total the average number of leaves present at the

beginning of the experiment was subtracted. The remainder represented the number of compound leaves added after the beginning of

FIG. 8.—Longitudinal section through node 15 of experimental plant collected at time differential light treatments were started. First morphological changes in response to short photoperiod subsequently found at this node on experimental plants. *l*, leaf base; *s*, stem tissue; *b*, primordium of bud which makes first response to photoperiodic stimulation.

treatment (table 2). Only those photoperiodic treatments which were continued more than 6 days were effective in modifying the course of

development of the apical meristems of the primary axis, and then only in the plants in series D to G, inclusive. Even in these the growing points formed a few additional primordia of compound leaves before differentiating any bracteal ones.

Reliable data were not obtained on the behavior of the growing points of side branches. The observations indicate that they may respond to treatments of somewhat shorter duration than do the main stems.

TABLE 2

TOTAL NUMBER OF COMPOUND LEAVES ADDED AFTER BEGINNING OF TREATMENT WITH SHORT DAY

Series	Number of days treated							
	1	2	3	4	5	6	8	10
A	17	18	17	17	17	15	11	.....
B	17	19	18	17	17	17	18	15
C	16	16	17	15	18	18	17	15
D	19	19	19	19	18	18	14	3
E	19	19	18	18	18	16	7	3
F	19	16	18	18	18	16	4	3
G	19	18	15	17	16	16	6	3
H	18	18	18	18	16	18	18	19

Conclusions

In many studies of photoperiodism, particularly those making use of the photoperiodic after-effect, apparently little attention has been given to the condition of the terminal meristems at the beginning of the experiment and during its progress. The effectiveness of the treatment has been judged at the time of flowering by various characters which are manifest at that time. As a consequence there is no way to determine whether the treatments that were effective caused initiation of flower buds or merely accelerated development of those already present. In the treatments where no effect was noted the plants may have made no visible response whatever, or they may have initiated flower primordia that failed to reach maturity under the conditions of photoperiod.

In experiments that attempt to explain the mechanism of the photoperiodic response of plants it seems necessary to know the

complete morphological development. Time of occurrence and localization of the first changes should be known. In this work with the soy bean, localized morphological responses resulting from two short photoperiods have been observed. It seems reasonable that these changes in morphology are preceded by or associated with changes in physiology brought about during the two days' treatment. If this is true, it seems desirable for the biophysicist and biochemist to give special attention to this early period. By so doing they will be studying the tissue at the critical time before other correlated effects, which may accompany differentiation, are expressed. Such investigational procedure on the biophysical and biochemical phases of photoperiodism is being pursued in this laboratory, and the results will be reported later.

Summary

1. Young Biloxi soy beans were grown for about a month on a long photoperiod. These long-day plants were divided into eight series, each receiving a different short photoperiod. Plants from each series were returned to natural day after 1, 2, 3, 4, 5, 6, 8, and 10 days.

2. Stimulation of two short photoperiods was sufficient to alter the course of development of the growing points in such a way that differentiation of flower primordia resulted.

3. The length of the photoperiod influenced the time of blossoming, since the plants receiving intermediate photoperiods blossomed earlier than those receiving either the extremely long or short photoperiods used in this experiment.

4. The number of days that the plants received these various treatments influenced the time of blossoming. Plants treated for 8 days blossomed earlier than those treated for only 6 days.

5. Meristems of plants growing on long day are described and compared with the meristems of those growing on short day.

6. The first visible response to short photoperiod occurred in the axil of the fourth leaf primordium from the tip of the main stem and in a similar position in certain axillary buds.

7. The region of quickest morphological response at the time the treatment is applied is an undifferentiated meristem.

8. An after-effect of photoperiodic treatment expresses itself in the total number of compound leaves produced on the main stem.

9. Treatments of less than 8 days did not suppress the addition of compound leaves.

10. Biochemical and biophysical studies which may throw light on these phenomena are in progress.

U.S. HORTICULTURAL STATION
BELTSVILLE, MARYLAND

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INDUCED PARTHENOCARPY[1]

FELIX G. GUSTAFSON

When a flower matures, the usual thing is for pollination and subsequent fertilization, seed, and fruit production to occur, but sometimes this does not happen. At times there may be no pollination, but even then in rare instances fruits without seeds may be produced; or pollination may take place but no fertilization and no seed development, yet fruits may be formed. Such parthenocarpy is not at all uncommon in some genera. Thus we have natural parthenocarpy such as seedless grapes, grapefruits, oranges, cucumbers, bananas, etc.

It is difficult to determine who made the first attempts to produce parthenocarpy artificially, but we know that in 1902 MASSART placed dead pollen upon the stigma of an orchid and observed a slight growth of the ovary. In 1909 FITTING (1) published his results on artificial parthenocarpy in orchids. FITTING used dead and living pollen and pollen extracts. He was able to cause some slight growth in the ovary of several species of orchids when the stigma was treated with dead or foreign pollen or drops of extracts. MORITA and LAIBACH repeated and verified some of FITTING'S work.

After many attempts to produce parthenocarpy by cross pollination, some of which were successful and others not, YASUDA (**7, 8, 9**) injected aqueous extracts of pollen into the ovary, with satisfactory results. Egg-plant ovaries injected with *Petunia* pollen extract grew to be 4.1 × 7.3 cm.; and in an experiment in which cucumber ovaries were injected with cucumber pollen extracts, three out of fifty ovaries grew, and at least one fruit measured 4.3 × 20.3 cm., which is the size of a normal cucumber. There were no seeds. Apparently, therefore, YASUDA should be given the credit of having first artificially produced parthenocarpic fruits.

In our laboratory at Ann Arbor, we have caused normal sized

[1] Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

parthenocarpic fruits to develop by treating the ovaries with a lanolin paste containing the residue of a chloroform extract of pollen. Extract from the pollen of *Petunia* caused egg-plant fruits to develop to a size of 2 × 7 cm., and they were still growing at the time of harvesting. *Petunia* pollen extract also caused parthenocarpy in pepper. Hubbard squash pollen extract caused cucumber ovaries to enlarge, and one large fruit and several smaller ones were produced (**4**).

Perhaps the most interesting work dealing with parthenocarpy is the production of fruits by treating the pistil with known chemicals, first accomplished at Ann Arbor (**3**). The use of known chemicals has much greater possibilities of supplying information concerning the mechanism of fruit development than has the use of pollen extract. It has been necessary, however, to show that pollen contains growth promoting substances.

In our earliest work we used indolepropionic, indoleacetic, indolebutyric, and phenylacetic acids mixed into a paste with lanolin. This paste was smeared either on the stigma or on the cut curface of the style. The first plant employed for this work was the tomato, and the first mature fruits were obtained in the late winter of 1936 with phenylacetic acid. The fruits were externally normal, but some were solid tissue with no trace of locules or seeds, while others had well developed locules but no seeds. Tomato fruits were also produced with the other compounds tried. Besides the tomato, fully developed fruits, without seeds, were also obtained in *Petunia*, *Salpiglossus*, and pepper; and egg-plant fruits were growing nicely when they had to be picked in the fall. Other plants were also used, and in some there was considerable growth, although full grown fruits were not produced.

Early in 1937, HAGEMANN (**6**) reported the production of parthenocarpy by treating flowers with indoleacetic acid. He secured full sized fruits, without seeds, in the gladiolus, the only plant tried in this experiment. GARDNER and MARTH (**2**) have introduced a new procedure which promises to be valuable. They sprayed pistillate flowers with various concentrations of the growth substances, using indoleacetic, indolepropionic, indolebutyric, and naphthalene acetic acids, in concentrations from 0.1 to 0.0001 per cent. They found the

naphthalene acetic acid to be the most effective. The plants with which they obtained success were holly and strawberry; no success was secured with apple and grape. GARDNER and MARTH also had success by watering the soil around the roots of the holly plants with 0.15 per cent indoleacetic acid solution.

For the last year we have continued this work at Michigan, and have found that injecting the material into the ovary through the pedicel is very effective in some plants like the tobacco. We have also tried the potassium salt of indoleacetic acid, and have found that in tobacco at least it is more efficient than the acid itself. This may be due, partly at least, to the fact that the salt is more soluble and a higher concentration could be used (1:500) as compared with 1:1000 of the acid.

In order to obtain additional information concerning the mechanism of fruit development, an entirely different set of experiments has been conducted (5), employing the crookneck summer squash, in which the ovules are located only at the apical end of the ovary. Although the lower end of the ovary contains no ovules, its growth rate in length is the same as for the rest of the ovary. A number of young fruits produced by pollination were cut off in such a way that only a few or no ovules were left. It was found that a considerable number of these grew, but all the fruits had some developing seeds in them.

In another experiment the ovaries from unopened flower buds were cut like the young fruits just mentioned, and the cut surface smeared with 5 per cent indolebutyric, indoleacetic, or pyrroleacetic acid. Most of the fruits treated with the first compound grew. It was very noticeable, however, that the ovaries which grew the most had some ovules present, although those ovaries from which all of the ovules had been removed grew too. This experiment tends to show that even the ovules themselves without the embryos stimulate growth in the ovaries where there has been no fertilization.

To discover whether a smaller number of seeds than normal would produce full sized fruits, experiments were performed with crookneck summer squash and pumpkin in which different amounts of the stigma were removed before pollination. The stigmatic surface was reduced by cutting off some of the lobes. In some experiments as much as five-sixths of the total stigmatic surface was removed be-

fore the pollen was applied by hand. The pollination was thus one-sided. Most of these ovaries grew into normal appearing fruits. Even though the number of seeds was much smaller than usual, their distribution was uniform. Thus reduced pollination which took place at one side of the ovary caused no change in the developing fruit, except that there were fewer seeds; but the number of seeds was sufficient for the production of a normal fruit.

From the experiments cited it is apparent that the pollen grain contains a growth promoting substance, which may be carried by the pollen tube to the ovary and cause it to grow; also the ovules may contain growth hormones, which may stimulate growth. Nevertheless, from our usual experience that unless fertilization has taken place and seed formation initiated there are no fruits formed, we are justified in believing that growth hormones are synthesized by the developing seeds, perhaps by the embryo. Sometimes, as in natural parthenocarpy, the ovules and perhaps the ovary itself may produce an unusually large amount of growth hormones, and the ovary grows into a fruit without fertilization first having taken place and no seeds are formed.

That fruits can be produced artificially by supplying growth promoting substances to the ovary has now been amply demonstrated. Whether this method will have any practical application remains to be seen. GARDNER and MARTH'S method looks promising for some plants. We have repeatedly tried to produce parthenocarpic fruits in summer and winter squashes, pumpkins, and watermelons with various growth promoting substances, but so far without success.

DEPARTMENT OF BOTANY
UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

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MERISTEMS AND FRUIT BUD FORMATION IN RELATION TO GENERAL HORTICULTURAL PRACTICE[1]

J. H. GOURLEY

Introduction

In considering the meristematic areas at the free extremities of shoots; that is, buds or bud potentials, the horticulturist has his ultimate concern with field practices which bring about or inhibit flower formation with consequent fruit setting and development. He finds it of interest to know the causal agents or factors involved, but in the end he must manipulate the environment of the plant or apply practices which will accomplish his purposes. It should not be inferred that the horticulturist is not interested in the details, nor that he asks his colleagues in other fields to work out the solutions of his problems; the converse of this has been amply demonstrated. It does remain patent, however, that the mass of detail must be brought together and applied by the plantsman, who feels the responsibility of a great industry.

The earlier fixed concept of the genetic constitution and expression of a given variety or species of plant has given way to one of great plasticity; that is, we have come to realize, all too slowly, that if we are greatly to change one or more of the important factors of the environment, then we cannot predict with any certainty the morphological results. The unlimited potentialities of protoplasm seem literally beyond our present comprehension; some new behavior arises of which we were unaware. It should be understood that this paper presents some of the more practical applications only, and attempts to indicate some of the practices designed to initiate flower primordia which are in vogue with the commercial fruit grower, although the list is far from complete. The literature is so voluminous that little attempt has been made here to cite particular reference or authority.

[1] Paper delivered before joint session of the Physiological Section of the Botanical Society of America, the Society of American Plant Physiologists, and the American Society for Horticultural Science.

Flower bud vs. fruit bud formation

There is much confusing terminology in use among horticulturists. Since the pomologist thinks in terms of fruit production, he has long referred to flower buds as "fruit buds." True this is only a term, but the implication is erroneous. It may be useful, however, in indicating trees grown for their fruit in which there are over-wintering flower buds as contrasted with annuals, succulents, and the like. But surely a clear cut distinction should be drawn between flower bud differentiation and fruit setting. Complete flower parts may and often are laid down by the meristem and anthesis reached, and yet few or no flowers develop into fruits. The factors involved are often quite different.

Cytological studies and fruit setting experiments in various places have shown very definitely that abundant flower production is no guarantee of fruit production. For instance, there are many ornamental forms of *Prunus* which flower abundantly, but because of their unbalanced chromosome situation are non-fruitful.

Relation of flowering to morphology

In the writer's opinion we can scarcely overstate the importance of being variety conscious in dealing with horticultural subjects. Varieties vary in their morphological detail as well as in their physiological responses to a given set of stimuli. To class all apples, or chrysanthemums, or what not, together, puts us in at least as disadvantageous a position as the plant breeder when he followed "mass selection." Then we must also learn that individuals of a given variety vary in their behavior because of differences of environment.

In referring to morphological differences of varieties, of which there are many, we may refer to the Rome Beauty apple for instance, which characteristically forms flower buds at the termini of shoots, and hence is an annual bearer. The Golden Delicious forms a large number of axillary flower buds when the trees are young, and hence has the machinery for annual flowering. Many varieties produce most of the flower buds on spurs and are much more likely to be alternate. Some, as the Livland Raspberry, develop comparatively few spurs, form few terminal or axillary flower buds on shoots, and hence are unreliable and low producers of both

blossoms and fruits. These structural situations cannot be ignored in a study of flower production of apples.

In the case of the peach and similar fruits, there is less of a problem of flower formation, owing to the occurrence of abundant lateral flower buds. It is mostly a matter of low temperature or other extraneous agencies limiting crop production.

Another morphological situation brought about as a result of some physiological response at the meristem is seen in the "everbearing" strawberry as contrasted with the conventional type. The latter produces flower buds during the long, warm days of summer, and shortly scapes arise, blossom, and fruit. The former forms overwintering ones during the shorter, cooler days of autumn. The buds in axils of the leaves of everbearers are mostly of flowers; hence few, if any, runners are formed. If the flower buds are removed early in the season, however, vegetative ones are formed, followed by runner formation, amounting to an increase of as much as 500 per cent in some cases, according to WALDO (**8**).

Yet despite these structural situations with which the horticulturist must deal, they must not be thought of as immutable, for it is this very plasticity of plant material which has given us a broader concept of the genetic expression of plants under varying environmental conditions. When we see a "carpel" terminated by an anther, a "stamen" bearing megaspores or appearing as a petal, and hear of fruits without flowers, it presents a challenge to anyone holding a static concept of plant parts.

Special practices affecting the apical meristem

The horticulturist must be concerned, wittingly or unwittingly, with all factors involved in flower bud formation which can in any way be controlled. Moreover, as stated earlier, he must consider their interrelation to all practices which enter into the operation of a garden or orchard. He may wish to induce the meristems either to initiate flower primordia or to avoid it. The next step, that of fruit setting, is of equal importance but is not to be confused with the former.

But before referring to some of the practices employed by orchardists, it would be pertinent to remind ourselves that the flower buds of the deciduous fruit trees of the north are initiated the

season before they bloom. The first evidences are usually found shortly after terminal growth ceases, late in June or early in July. The duration of the period during which the floral primordia are differentiated may extend over two months or slightly longer, but the peak occurs during July. It has been shown by MAGNESS and coworkers that the period during which this can be influenced in the apple extends for about 45 days from full bloom, but the earlier the better. We are indebted to GOFF of Wisconsin for the first studies of this sort in America, at the beginning of this century, but much confirmatory work has been done by a number of others. This is mentioned because practices designed to influence this phenomenon must be timed in accordance with this knowledge.

As one surveys the field of specific practices, he is baffled as to which to cite because of their number. Among such practices or situations associated with flowering are the following:

1. Selection of variety. That variety which exhibits a strong tendency to flower regularly and freely and is otherwise desirable contributes more to a successful enterprise than all the artificial devices put together.

2. Practice of maintaining a high and uniform state of "vigor" of the trees, in which neither carbohydrates nor organic nitrogen nor nutrients are limiting. Such trees are the most regular in producing flowers and fruits, barring frosts and other hazards.

3. Girdling of the trunk or branches. Damage by fire, rodents, disease cankers, winter injury, or other injuries of this class would fall in the same category.

4. Bending of branches out of their upright position, thus contributing to an early cessation of growth extension.

5. Root pruning, which reduces the uptake of moisture, nitrogen, and other nutrients.

6. Thinning of the fruit.

7. Use of dwarfing, or semi-dwarfing, stocks.

8. Low water supply at time of initiating floral primordia.

9. Abundant leaf surface.

Some situations which are likely to limit flower formation are:

1. Excessive vegetative condition at the critical time, resulting in a carbohydrate and possibly hormone deficiency during the time of flower initiation.

2. Heavy pruning which stimulates growth extension at the expense of flower production.

3. Shading.

4. Defoliation, or injured foliage, from whatever cause.

Space will not permit a discussion of all of these factors, but a few may be mentioned in more detail.

NITROGEN AND ORCHARD CULTURE.—These two items may be treated together because available nitrogen and soil culture are closely associated in the mind of the orchardist. Nitrogen has been the element most commonly and successfully applied for the purpose of fruit production. But there is no close relationship between the amount of available nitrogen in the soil and flower bud formation. This is very different with fruit setting and development. True it has been shown by nearly all workers in this field that there is a high concentration of organic nitrogen in flowering as compared with non-flowering spurs. But frequently low-nitrogen trees bloom profusely only to lose practically all the flowers shortly after petals fall.

As one finds trees in either extreme of vegetation he sees a strong tendency toward failure of flower buds to develop, or if they develop to fail to set fruit.

In some unpublished work, the writer has found that 16-year-old Baldwin and Stayman Winesap trees, which have been treated with three times the usual amount of nitrogen, have fewer spurs forming flowers than when more moderate amounts were used. The results of similar experiments elsewhere have been so diverse that it has become apparent that it is the soil and culture that determine the fertilizer requirements, rather than the kind of fruit.

Some earlier work (1) showed that both peach and apple trees shaded with cheesecloth failed to produce flower buds as abundantly as those exposed to full sunlight, thus illustrating again that low carbohydrate-high nitrogen trees are not fruitful. Yet in all the cultural work which has been under my observation, the trees supplied with ample moisture and nitrogen were the most regular producers of flowers and fruits. The treatment to be applied depends on the starting point or the internal condition of the trees.

GIRDLING.—Ringing is the most commonly employed artificial means of bringing about flower bud formation in the apple and pear. It results in an accumulation of carbohydrates and a reduction of

nitrogen, together with other physiological phenomena, above the wound, which are associated with flower bud formation. It might be said that the effects of ringing are somewhat similar to those of root pruning, or to a sudden shift of vigorous trees to sod culture, low nitrogen supply, or more remotely to lack of pruning, or any other abrupt check in growth.

It is unnecessary to cite data to show that the removal of a ring of bark or the "scoring" of trees, or any other such injury, is likely to result in abundant flower formation, and such an observation was doubtless in part responsible for the old notion that "a tree tries to reproduce itself just before it dies." But here too, certain conditions must be met as to vigor, age, and size of tree as well as variety. For a time this old practice was unpopular, but it is now used to a limited extent in commercial orcharding, especially with "filler" trees.

THINNING.—Removal from a tree of a portion of the fruits before they are mature, usually when quite small, relieves competition among them, improves size, color, and quality, and influences regular bearing. For the last purpose, however, the practice has proved a great disappointment.

More recent work by the U.S. Department of Agriculture (6) has shown that earlier and heavier thinning had a most pronounced effect in increasing flower formation, even with such obstinate varieties as York Imperial and Yellow Newtown, provided the trees were vigorous and there was a high leaf to fruit ratio. In the case of Yellow Newtown, if the work was done 37 days after full bloom with seventy leaves (or more) per apple, 47 per cent of the spurs formed flower buds. If thinning was done at the same time but the leaf-fruit ratio was fifty, then only 23 per cent flowered. If the thinning was done 73 days after full bloom very few spurs flowered, regardless of number of leaves, thus emphasizing the importance of early thinning. Thus alternate bearing trees become annual ones, or if the thinning is neglected after the first year, the trees again alternate but with the crop year reversed. This is a new trend and has not as yet been put to the practical test on a commercial scale. The expense involved is a partial obstacle.

Such results would formerly have been associated entirely with conservation of food, but now the role of hormones must be con-

sidered as a second operating factor, although data on this point are lacking so far as the writer is aware.

WATER.—The exact role of water in this connection is not so easy to define. That water is essential for plant growth to the extent that we think of both the soil medium and the plant cells as bathed in it, is an old concept. Water is essential for flower formation of course; yet it is after a year of extreme drought that we see all sorts of perennial woody plants flowering in great profusion. This is particularly true if the water shortage is acute early in the summer. All this sounds paradoxical. If experimental evidence is examined, however, we do not find that irrigation during a dry season increases the flower crop in the ensuing year unless the trees had actually become devitalized, according to MAGNESS (**5**) and others. Apparently there is no close connection between amounts of water available and behavior of the meristem, as is true of inorganic nitrogen.

PRUNING.—Without attempting a discussion of pruning, it may be said that heavy pruning of young trees results in a marked delay in flowering and fruiting, as well as in a smaller tree, although the length of growth near the points of the cuts is increased. Practices current less than a quarter of a century ago are now practically reversed. The earlier type of pruning employed increased the ratio of leaf bud-flower bud formation, and also removed too much potential bearing surface. In other words, the whole tendency is toward lighter pruning.

Factors involved in flower bud formation

It is not possible to discuss all the practical applications in a paper of this length, but it is hoped that sufficient have been cited to indicate the diversity of the problems encountered. One or two general observations may now be made by way of summary. Throughout, two factors are prominent: (1) the need for an abundance of "healthy" green leaves, whatever they produce; and (2) a slowing or checking of growth just prior to flower bud differentiation.

Since the earliest records of man's interest in the reproductive processes of plants, there is evidence of varied speculation as to the basic factors involved. But it has been within the active period of most of us that a workable hypothesis was proposed to account for

the vast array of phenomena of reproduction. The researches and proposals relative to carbohydrate-nitrogen relationships explain more situations than any others. True they do not seem to account for all, because of our as yet incomplete knowledge, and because still other factors are involved. They have, however, given to the horticulturist for the first time a usable tool, a way of thinking of plants which can be understood and manipulated by an intelligent plantsman.

MURNEEK (7) has recently pointed out that the reverse of this situation obtained in the initiation of flower primordia in Biloxi soy beans, and it is quite likely that we cannot generalize from one species or genus to another, so far as the exact details are concerned; but in the end we are likely to come back to the necessity of an adequate food supply within the plant as a factor of major consideration.

Other more specific substances than carbohydrates and organic compounds are receiving attention as the primary causal agent of flower initiation, and interesting evidence is at hand; but in any event it is in the green leaves or other green organs that these substances find their origin. The horticulturist need not at this time quibble over nor greatly concern himself as to whether some hormone or flower promoting substance is more important than the food constituents in accomplishing the desired results. The fact still remains that flowers and their end products—fruits and seeds, are composed largely of the products of photosynthesis and of organic nitrogen complexes. It is the green leaf, unharmed by insect, disease, caustic spray soiution, or other extremes of environment, that tells the story. That is, these things come within the range of the practicing horticulturist.

Of special importance is the discovery that lime-sulphur solutions reduce the rate of photosynthesis and yield of fruit in subsequent years when used at certain concentrations. A dilution of one gallon to forty of water, which was formerly a standard strength for summer application, results in reduced CO_2 absorption, according to HOFFMAN (2, 3, 4), although no injury to the foliage may be apparent. When more dilute solutions are used the objection is largely obviated.

In the end, however, it is an abundance of functioning green foliage that is the key to the situation; without it other practices will avail little.

But after all, this is largely a matter of studying factors which are limiting, either inside or outside the plant. These factors may be soil nutrients, water, food supply within the plant tissues, catalysts (organic or inorganic), light, temperature. If something like an "ever-normal granary" could be maintained within the perennial woody plant, the cycles of flowering and fruiting would be less conspicuous or absent. It is because the orchardist permits exhaustion of the food reserves, is ignorant of or neglects provision for nutrient or catalyzing substances, selects unfavorable soil and site, or fails to maintain healthy foliage that there is failure of flower or fruit production.

Finally, anything that slows down or checks growth at the proper time usually results in the meristem laying down flower parts. This may come about with or without the interference of man.

OHIO EXPERIMENT STATION
WOOSTER, OHIO

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CORRELATION OF CAMBIAL ACTIVITY WITH FLOWERING AND REGENERATION

OCRA CHRISTINE WILTON

(WITH THIRTY-SEVEN FIGURES)

Introduction

In a previous publication, WILTON and ROBERTS[1] reported that a decrease in cambial activity in the higher internodes of certain herbaceous dicotyledons accompanies the production of flowers, but data showing that this condition may extend to the lower levels of the stems were not presented at that time. Information concerning the degree to which cambial activity throughout a stem is correlated with the reproductive condition is of interest as a means of eliminating the question of age of plant, which is frequently offered as an explanation of the anatomical characteristics sometimes associated with flowering. It is of further interest in its possible relation to the ability of some plants to continue or renew vegetative growth after flowering while others die at the close of the first flowering cycle.

Material and methods

In order to determine the extent to which the cambial condition throughout the plant is correlated with the production of flowers, a study was made of cross sections of all internodes from the tips to the bases of stems of comparable flowering and non-flowering plants. The allied question of the relation of cambial activity to regeneration was investigated by examining sections from internodes both above and below second growth shoots on plants which had already passed through one flowering cycle.

From a study of more than two dozen species, the following were selected as representative for this study: *Cannabis sativa* L.; *Amaranthus retroflexus* L.; *Dianthus chinensis* L. var. Heddewigii; *Delphinium cultorum* Voss; *Sidalcea parviflora* Greene; *Chrysanthemum*

[1] WILTON, OCRA CHRISTINE, and ROBERTS, R. H., Anatomical structure of stems in relation to the production of flowers. BOT. GAZ. **98**:45–64. 1936.

morifolium Ran. var. Lillian Doty; *Cosmos sulphureus* Cav. var. Klondike.

Material of *Cannabis* was grown in the University greenhouses during the winters of 1935–36 and 1936–37. Since this is a dioecious species, three groups of samples were taken: (1) From plants which had grown in a short-day house at 70° F., where they attained a height of more than 6 feet and were in a vigorously vegetative condition with no indication of floral buds at the time of sampling. The basal internodes were approximately five-eighths of an inch in diameter. (2) From plants which had produced pistillate flowers and at the time of sampling were bearing immature fruits. (3) From plants which had produced staminate flowers. The latter two groups were grown in a short-day environment in a house kept at approximately 60° F. For this species, the highest samples were taken from the first internode below the raceme and others from each successively lower internode to and including the hypocotyl.

The material from which samples of *Amaranthus* were taken was grown under a variety of environmental conditions in order to obtain varying degrees of reproductiveness and vegetative vigor. One group of plants was grown at a minimum temperature of 60° F. in a short-day environment. Under such conditions all of the primordia, both terminal and axillary, throughout the length of the stem differentiated as floral buds. There were no lateral branches. Such plants were termed extremely reproductive. A second group of plants was grown at 70° F., in a long-day environment, where they branched freely toward the base of the stem, required a longer time to flower, and differentiated relatively fewer floral buds than the plants grown in the cooler short-day environment. These plants were termed moderately reproductive. Plants grown in the long-day house at 60° F. were strictly vegetative, no floral structures being differentiated in the period of four months during which they were observed before sampling. Samples taken from the internode directly below the terminal inflorescence in the case of the flowering plants were designated as "first," and those from the lower internodes were numbered consecutively. The highest samples were taken from the fourth elongated internode of the non-flowering plants and also from the consecutively lower internodes. A fourth

group of *Amaranthus* plants was grown in a short-day house at 60° F. from the time of germination until a terminal inflorescence in an early stage of development could easily be recognized. The plants were then given a long-day environment although still kept at 60° F. Here development of the floral buds continued and seeds were produced, but no new floral buds were differentiated and vegetative branches were developed from the axillary buds below the terminal inflorescence. Samples were taken from internodes both above and below the new branches.

The plants of *Dianthus*, *Delphinium*, and *Sidalcea* were grown in the horticultural garden during the summer of 1936. All three species produced flowers, and at the time of sampling had developed second growth, vegetative branches. In the case of *Dianthus* the branches usually came from the second and third nodes above the ground, while the second growth branches of *Sidalcea* developed from the nodes about midway between the tips and bases. Samples of both species were taken from the internodes both above and below the new branches. The vegetative shoots of *Delphinium* came from the base of the plant just below the surface of the ground. For this reason samples were not taken below the new branches, but were taken from the basal internode of the stem and from the internode just below the inflorescence.

For the material of *Chrysanthemum* and *Cosmos*, two lots of plants were grown. One had only the light of the normal short days of autumn and in this case flowers were produced. The other lot was given an additional eight hours of artificial illumination and did not become reproductive. Photoperiod was the only environmental factor which was varied for these two species. Samples were taken from the first internode below the peduncle and from each successively lower internode of the flowering plants. Sampling was delayed until the reproductive condition was far advanced, particularly in *Cosmos* where there were numerous lateral flowering branches. The highest samples taken from the non-flowering plants were from the fourth elongated internode. The higher internodes are frequently too immature to show significant differentiation of secondary tissues.

The fresh samples were killed and fixed in formalin-acetic-alcohol.

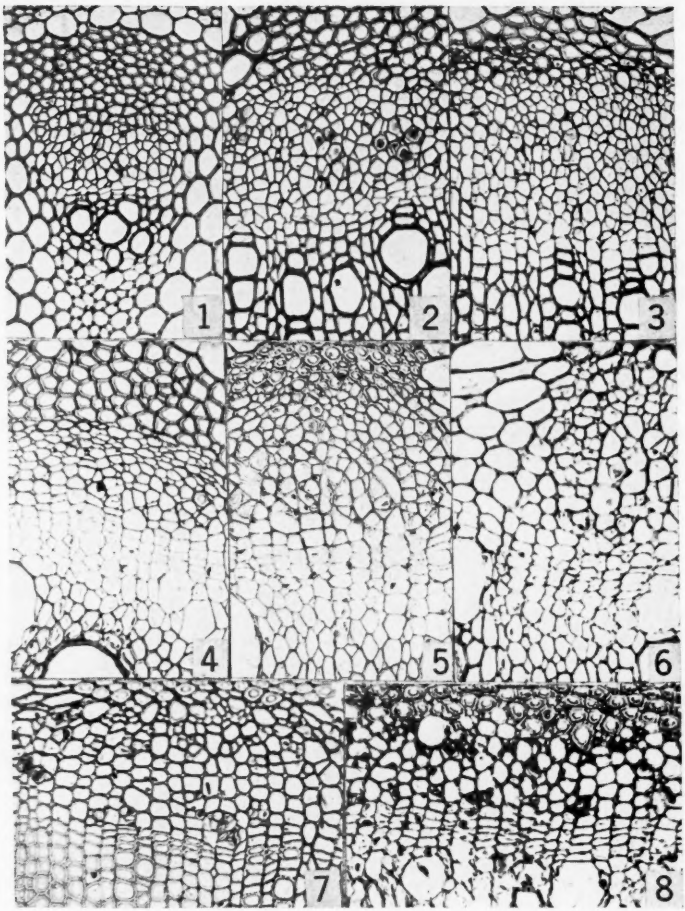
Butyl alcohol was used for dehydrating and the material was imbedded in paraffin. Sections 15 μ in thickness were made and stained with a combination of safranin and light green.

Observations

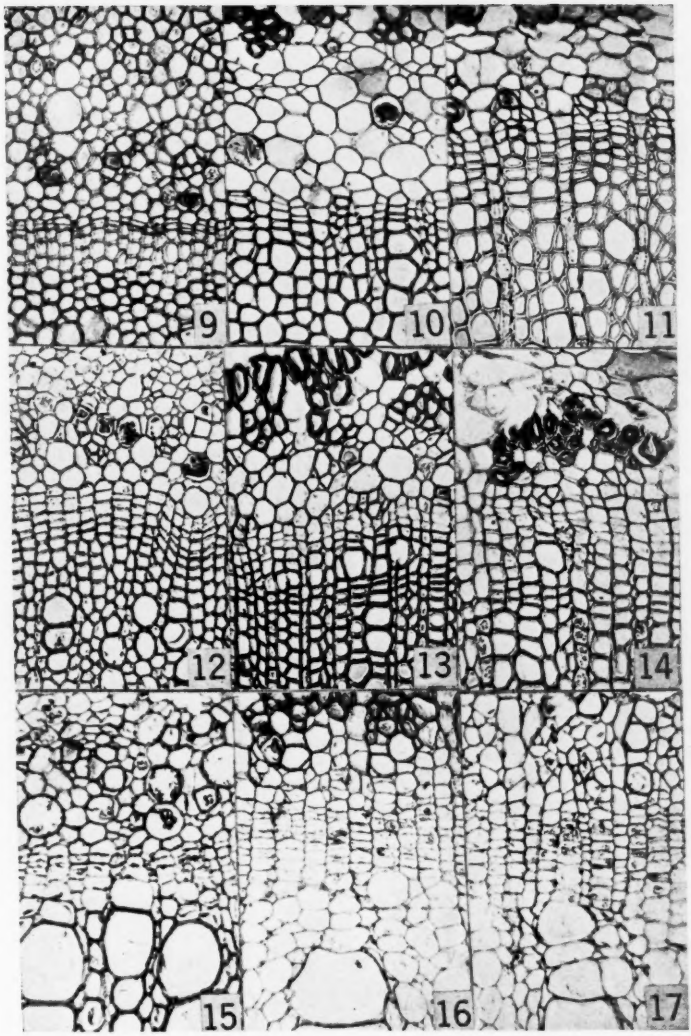
There is little or no cambial activity in any part of the stem of an abundantly flowering plant of *Cosmos* (figs. 1–3). In contrast to this condition, the non-flowering stem shows an active cambium throughout its length (figs. 4–6). The condition in *Chrysanthemum* so closely parallels that in *Cosmos* that only the basal internodes are figured (figs. 7, 8).

The slight difference in degree of maturity between the male and female plants of *Cannabis* (figs. 9–14) corresponds to their difference in vegetative vigor, which was apparent from external observation of the plants. The male plants died soon after the pollen was shed while the female plants remained green until after the seeds were mature. The stems of *Cannabis* also illustrate another characteristic which has been observed in all the species so far examined having a racemose inflorescence. Sections from the first few internodes directly below the inflorescence have relatively small cells and a weakly vegetative appearance. For this reason sections from the middle region of the stem usually give a better indication of the reproductive condition of such plants, as is illustrated by a comparison of figures 10, 13, and 16 with figures 9, 12, and 15.

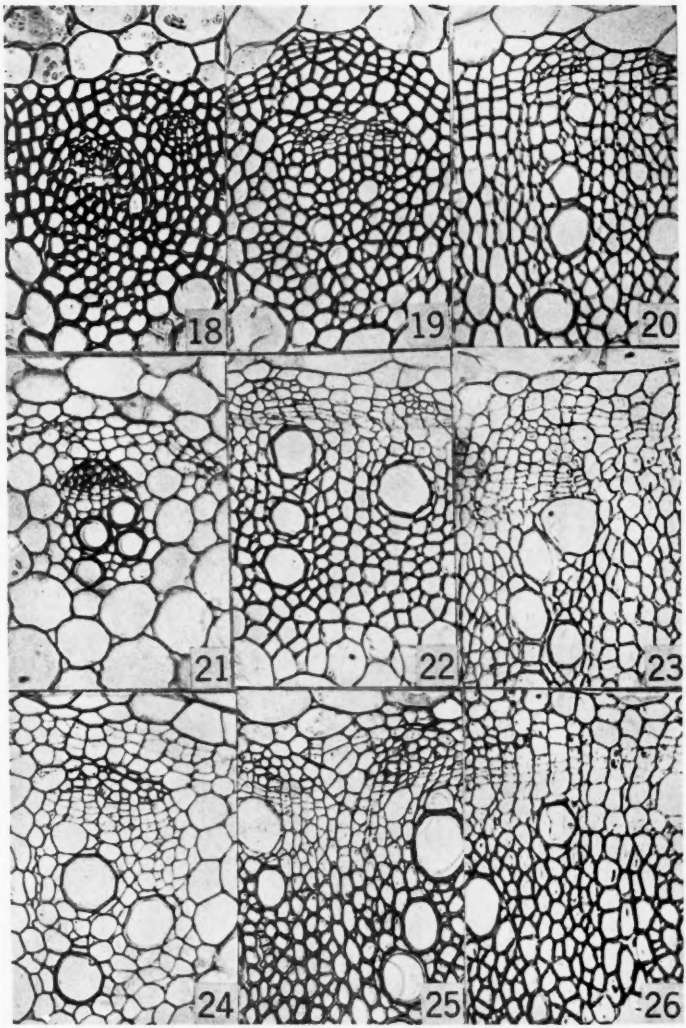
The material of *Amaranthus* (figs. 18–26) also illustrates quantitative variations in degree of maturity of meristematic tissue. In this instance the variations in degree of reproductiveness were induced by differences in environmental conditions, and were evident in the external appearance of the plants. It should be noted that there is a complete absence of meristematic tissue in the stems of the extremely reproductive plants (figs. 18–20). However, a progressive decrease in thickness of the cell walls from the tip toward the base of the stem indicates a basipetal sequence in maturity corresponding to the time of cessation of cambial activity. The sharp contrast between these sections and those from the non-flowering stem (figs. 24–26) is somewhat modified by the sections from the moderately reproductive plants (figs. 21–23) which have some tissue that is at



FIGS. 1–8.—*Cosmos:* Figs. 1–3, transections from second, seventh, and tenth internodes of flowering plant showing little or no cambial activity even in basal internode which was just above the hypocotyl. Figs. 4–6, comparable sections from fourth, eighth, and twelfth internodes of non-flowering plant showing active cambium throughout ler.gth of stem. *Chrysanthemum:* Fig. 7, from twenty-fourth internode of flowering stem; all elements appear to be differentiated and mature, no elements resembling cambium being present. Fig. 8, from twenty-eighth internode of non-flowering stem where there is active cambium.



FIGS. 9–17.—*Cannabis:* Figs. 9–11, sections from tip, middle, and basal regions of stem of male plant; no meristematic tissue at any level. Figs. 12–14, from stem of female plant showing a lesser degree of differentiation of cambial elements than figs. 9–11, although the only evidence of cambial activity is in the basal internode. Figs. 15–17, from non-flowering plant: 15, from fourth elongated internode, too immature to have differentiated much secondary tissue; 16 and 17, from middle and basal regions, showing active cambium.



FIGS. 18–26.—*Amaranthus:* Figs. 18–20, transections from first, seventh, and thirteenth internodes of extremely reproductive plant; complete absence of meristematic tissue and relatively large amount of mechanical tissue noted. Figs. 21–23, comparable sections from moderately reproductive plant showing more or less potentially meristematic tissue which increases progressively from tip to base. Figs. 24–26, from fourth, eighth, and twelfth internodes of non-flowering plant; amount of cambial activity markedly greater in figs. 24 and 25 than in figs. 21 and 22, where flowering condition is distinctly evident. Little difference in figs. 23 and 26.

least potentially meristematic, but with decidedly less cambial activity in the higher internodes than in the non-flowering stem.

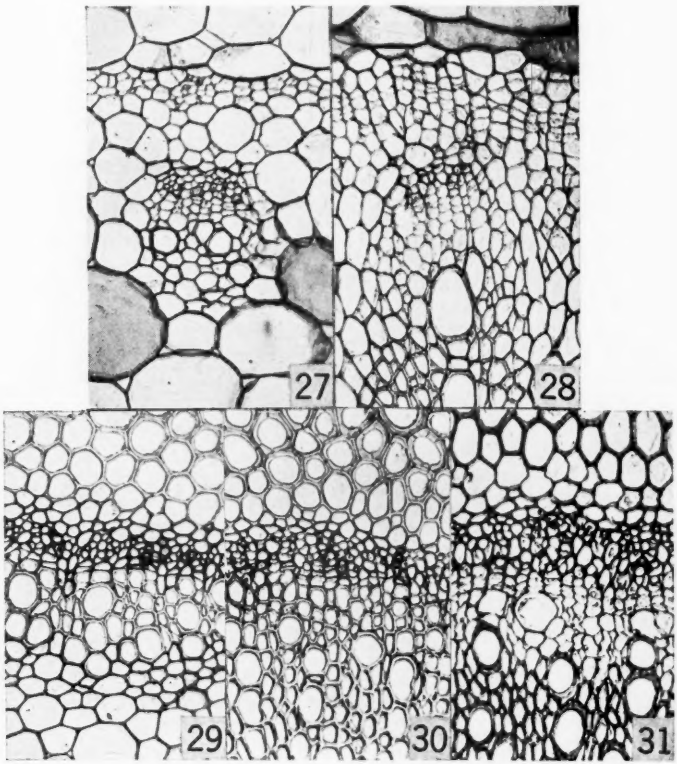
The greater degree of "maturity" of the higher internodes in contrast to the condition of the basal internodes which accompanies the differentiation of floral structures is to be interpreted as proof of the correlation of anatomical structure with flowering rather than with chronological age. Here the youngest internodes are first to become mature. Further proof is to be seen in the stems of the non-flowering plants where the opposite situation seems to prevail. These plants have a greater amount of meristematic tissue in the higher internodes than at the base of the plant.

It has been pointed out that certain species which differentiate floral buds in abundance, as do *Cosmos* and *Amaranthus* when grown at 60° F. in a short-day environment, have no meristematic tissue remaining in any part of their stems at the time of full flowering. This phenomenon suggests a possible explanation of the fact that these plants usually die at the close of one flowering cycle. It would seem that there are no elements remaining which are capable of dividing, and thus a renewal of growth is not possible even though environmental conditions may have become very favorable for vegetative development.

On the other hand, there are plants which retain some vegetative growing points while flowering either continuously or in cycles throughout a season; or after the production of flowers and fruits may lose only the terminal internodes of their branches while second growth shoots are developing at a lower level of the stem. An explanation for such habits of growth is suggested by the variations in degree of cambial activity between the tip and the base of the stems which were noted in the moderately reproductive plants, for example, *Amaranthus* grown at 70° F. in a long-day environment. It was reasoned that any part of the stem in which cambial tissue remained, either active or potential, could continue or renew vegetative growth. An attempt was made to find some definite proof for this hypothesis by an examination of the internodes, both above and below vegetative branches, which had developed after a cycle of flowering.

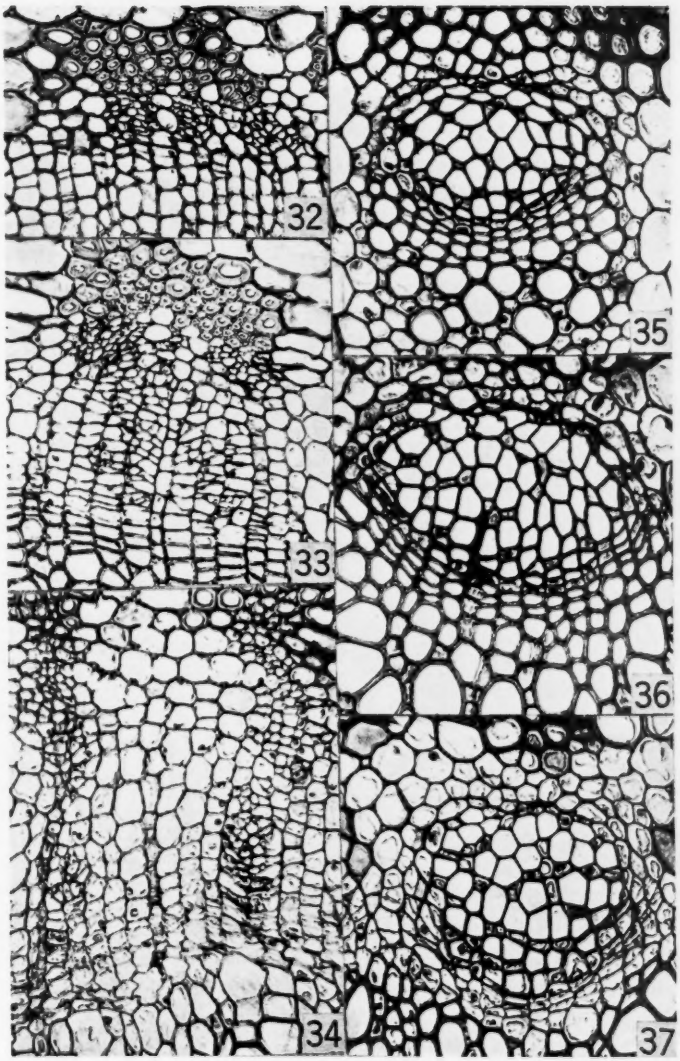
In *Amaranthus*, *Dianthus*, and *Sidalcea*, where the second growth

branches developed along the aerial stem, the internodes immediately above the highest branches have a slight amount of cambial tissue but never an active cambium (figs. 27, 30, 33). In *Dianthus*



FIGS. 27–31.—*Amaranthus:* Fig. 27, transection from third internode below inflorescence and above lateral, non-flowering branches; anatomical condition similar to fig. 23. Fig. 28, same stem below second growth, non-flowering branches. *Dianthus:* Fig. 29, below flowers; no undifferentiated tissue present. Fig. 30, same stem just above young vegetative branches; a few thin walled elements in cambial region. Fig. 31, below vegetative shoots, showing wide zone of meristematic tissue and numerous recently differentiated xylem elements.

and *Sidalcea* the higher internodes show complete maturation of the meristematic tissue (figs. 29, 32). In *Amaranthus*, where there were only three relatively short internodes between the inflorescence and



FIGS. 32–37.—*Sidalcea:* Fig. 32, transection from internode below inflorescence; no undifferentiated tissue remains. Fig. 33, from middle region of stem, above second growth branches; a few cambial elements present. Fig. 34, from basal internode below vegetative branches, showing wide cambial zone. *Delphinium:* Figs. 35, 36, transections from internode directly below inflorescence and basal internode respectively; no undifferentiated elements seen in either case. Fig. 37, characteristic structure of non-flowering stem.

the node from which two lateral vegetative branches developed, there is some tissue which is at least potentially meristematic even in the first and second internodes; but these appeared to have less vegetative vigor than the third which was photographed (fig. 27). In the case of *Delphinium* (figs. 35, 36), which did not have second growth branches along the aerial stem, the meristematic tissue was completely differentiated throughout the stem. The location of second growth branches was by no means constant for a given species, but an examination of numerous samples such as the ones figured indicated that a revival of vegetative activity is dependent upon the presence of at least a small amount of meristematic tissue. Conversely the highest second growth branches will indicate the approximate upward limit of cambial activity in the stem.

Summary

1. If certain species of plants are allowed to reach an advanced stage of reproductiveness under favorable environmental conditions, the meristematic tissue of their stems tends to become entirely differentiated into xylem and phloem elements. This anatomical condition is a possible explanation of the death of such plants at the close of one reproductive cycle.

2. The cessation or decline of cambial activity which accompanies the production of flowers progresses from the region of the inflorescence toward the base of the plant, which it may or may not reach depending upon the degree of reproductiveness which the plant attains as measured by the relative number of primordia which differentiate floral structures.

3. Vigorously vegetative plants have an active cambium throughout the length of their stems.

4. There appears to be no correlation between the chronological age of the internode and the decrease in cambial activity in plants which are producing flowers.

5. A certain amount of at least potentially meristematic tissue seems to be necessary for a renewal of vegetative growth in stems.

DEPARTMENT OF HORTICULTURE
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

NUCLEAR SIZE IN RELATION TO MEIOSIS

J. O. BEASLEY

The majority of papers devoted to meiosis limit the observations and discussion to chromosomes. No doubt chromosomes should have the most emphasis in a consideration of meiosis, but their pairing, coiling, and movement can be more adequately explained if some attention is given the nucleus and karyolymph within which the chromosomes operate. Any study of meiosis reveals a great increase in size of first prophase meiotic nuclei over prophase nuclei in somatic cells, and it usually gives evidence of a great change in the viscosity or density of the karyolymph in the prophase stages. Although size and viscosity changes in the nucleus have frequently been observed in studies of meiosis, little seems to have been done in the way of relating them to chromosome behavior.

Various theories have been advanced to explain the chromosome behavior observed during meiosis, especially as to the cause and mechanism of chromosome pairing. DARLINGTON (1) suggests that chromosome threads normally show a strong tendency to associate in pairs. The tendency is satisfied in mitotic prophase, because the threads are double as a result of a split that occurs in the resting stage before prophase begins. Pairing in meiosis is thought to result from the precocious initiation of the prophase before the chromosomes split, which makes it necessary for chromosomes to pair in order that the tendency to be associated in pairs be satisfied.

Some observations fail to fit this scheme. In *Diptera*, METZ (4) reports paired somatic chromosomes, and other writers have reported similar phenomena, some of which were in plants. Several workers have reported double chromosomes in the leptonema stage. In a study of *Drosophila*, KOLLER (2) found salivary chromosomes to be composed of more than one strand at the time they begin to pair. In such cases chromosome pairing must be attributed to something other than a tendency of single chromosome threads to be associated in pairs. STEBBINS (8) states that at leptotene each chromosome is

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Summary

1. If certain species of plants are allowed to reach an advanced stage of reproductiveness under favorable environmental conditions, the meristematic tissue of their stems tends to become entirely differentiated into xylem and phloem elements. This anatomical condition is a possible explanation of the death of such plants at the close of one reproductive cycle.

2. The cessation or decline of cambial activity which accompanies the production of flowers progresses from the region of the inflorescence toward the base of the plant, which it may or may not reach depending upon the degree of reproductiveness which the plant attains as measured by the relative number of primordia which differentiate floral structures.

3. Vigorously vegetative plants have an active cambium throughout the length of their stems.

4. There appears to be no correlation between the chronological age of the internode and the decrease in cambial activity in plants which are producing flowers.

5. A certain amount of at least potentially meristematic tissue seems to be necessary for a renewal of vegetative growth in stems.

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composed of four chromonemata, and he points out that one difference between meiosis and mitosis is the greater length of meiotic prophase. He offers a hypothesis that the physiological condition of the nucleus in early prophase of every mitosis promotes the coming together of chromonemata, and that in meiosis the prophase is prolonged. SAX and SAX (6) point out that there is no reason to suppose that the last mitotic division differs from the others. They suggest that meiosis results from a retardation of cellular activity, as indicated by the much longer time required for meiosis than for mitosis, the longer time permitting chromosomes to uncoil completely and to pair intimately with gene by gene association. This intimate pairing was envisaged as being prevented in mitosis by the comparatively rapid prophase, which prevents the chromosomes from ever completely uncoiling.

Later it was realized that an increase in size of the nucleus, as well as the longer time required for meiosis, could be a factor in allowing chromosomes to uncoil completely and to pair.

To show the constant occurrence of increase in size of prophase meiotic nuclei over somatic nuclei, measurements were made in millimeters of the diameters of nuclei in published drawings and photomicrographs showing meiotic and somatic nuclei (table 1). It is seen that in each case there is a great increase in size of prophase meiotic nuclei over somatic nuclei. In such illustrations the somatic nuclei were probably in the resting stage. In some cases the resting stage before meiosis, or earliest meiotic (early leptotene), is given, for it is shown in table 2 that these stages differ little from somatic prophase. PAUL (5) made measurements of prophase and resting meiotic nuclei. His data show the resting nucleus to average 7 μ in diameter, and prophase nuclei to average 11.2 μ. The largest diameter was at synizesis, and he states "It is very tempting to conclude that the activity of the nucleus is maximum during synizesis."

In order to estimate the size of resting and prophase nuclei in mitosis and meiosis, the diameters of nuclei were measured in several parts of *Gasteria* (table 2). The measurements were made from permanent slides of sectioned material.

Prophase nuclei in root tips were $1.48 \pm 0.149\ \mu$[1] larger in diam-

[1] Standard error.

eter, and they had a volume 1.44 times greater than resting nuclei in the same tissue. Somatic prophase nuclei in integuments were 0.51 ± 0.142 μ smaller in diameter than prophase nuclei in root tips. The resting stage after the last mitotic division before meiosis aver-

TABLE 1

COMPARATIVE MEASUREMENTS OF PROPHASE MEIOTIC NUCLEI AND SOMATIC NUCLEI FROM PUBLISHED DRAWINGS AND PHOTOMICROGRAPHS IN THE AMERICAN JOURNAL OF BOTANY

Author	Species	Meiotic (mm.)	Somatic (mm.)	Earliest meiotic (mm.)	Meiotic / Somatic
Pickett (1916)....	Arisaema	8.6	4.4	.....	1.95
Chipman (1925)...	Lilium superbum	50.0	.....	39.0	1.28
Sorokin (1927)....	Ranunculus acris	11.5	.....	7.4	1.55
Nevins (1927).....	Furcraea andina	4.5	2.5	.....	1.80
Cooper (1931).....	Lycopersicon esculentum	4.0	1.8	.....	2.22
Pastrana (1932)...	Begonia schmidtiana	27.0	.....	19.0	1.42
Cooper (1935).....	Portulaca oleracea	5.5	2.0	.....	2.75
Taylor (1931).....	Gasteria	17.7μ*	.....	14.0μ*	1.26

* Average diameters published by Taylor.

TABLE 2

SIZE OF MEIOTIC AND MITOTIC NUCLEI IN GASTERIA

Part of plant	Diameter (μ)	Volume (μ³)	Number
Resting in root tips	11.38±0.12	772	49
Prophase in root tips	12.86±0.09	1114	50
Prophase in integuments	12.35±0.11	986	38
Pre-meiotic prophase in anthers	13.86±0.15	1394	62
Pre-meiotic prophase in anthers*	13.53±0.13	1297	32
Resting before meiosis p.m.c.*	11.95±0.08	894	25
Beginning prophase p.m.c.*	13.78±0.10	1370	50
Prophase p.m.c.	19.05±0.21	3620	33
Prophase p.m.c.*	18.17±0.19	3141	34
Prophase megaspore m.c.	19.01±0.43	3597	28

* This material was imbededd at a different time from the rest, but received approximately the same treatment.

aged 0.57 ± 0.147 μ larger in diameter than the resting stage in root tips, and premeiotic prophase nuclei in anthers were 1.00 ± 0.171 μ larger than prophase nuclei in root tips. These differences show that premeiotic nuclei may be slightly larger than other somatic nuclei.

Prophase (leptotene to diplotene) nuclei in pollen mother cells were 6.22 ± 0.209 μ greater in diameter, and 3.52 times greater in volume, than the resting nuclei of pollen mother cells. The resting stage in pollen mother cells was only 1.16 times greater in volume than the resting stage of root tips, but the prophase of pollen mother cells is 3.25 times greater in volume than the prophase in root tips. Prophase nuclei of megaspore mother cells averaged 3.23 times greater in volume and 6.15 ± 0.439 μ greater in diameter than prophase nuclei in root tips. The volume of 894 μ^3 for resting pollen

TABLE 3

SIZE OF MEIOTIC AND MITOTIC NUCLEI IN ANIMALS*

MATERIAL	DIAMETER	VOLUME	NUMBER
Testis of mouse			
Resting somatic....	6.64	153	10
Resting meiotic....	6.25	129	10
Prophase meiotic...	10.06	533	10
Testis of grasshopper			
Resting somatic....	10.40	589	10
Prophase meiotic...	16.14	2202	10

* I am indebted to Professor L. Hoadley for the use of slides from which these measurements were made.

mother cell nuclei and the volume of 3141 μ^3 for prophase pollen mother cell nuclei correspond well with the volumes of 950 μ^3 and 3823 μ^3 given for *Gasteria* by TAYLOR (9). It is shown in table 3 that there is a correspondingly great increase in prophase meiotic nuclei in animals.

The increase in size of first prophase meiotic nuclei can hardly be attributed to increases in the quantity of stored food, for there is a lack of evidence of stored food in nuclei, and reproductive cells that possess large amounts of food, store it outside the nuclei. The increase in size must be a result, cause, or contributing factor in meiosis.

From most cytological preparations it appears that as the nucleus expands the karyolymph becomes more liquid, until the nucleus reaches its maximum size. The watery nature (low viscosity) of karyolymph in meiotic nuclei at zygotene is evidenced by the usual

collapsed condition of chromosome threads in preparations showing zygonema stages. From cytological observations it appears that as meiosis progresses from early zygotene, the karyolymph gradually thickens, no doubt from the diffusion of materials into it from the cytoplasm. At the end of diakinesis it seems reasonable that the osmotic values of karyolymph and cytoplasm are equal, and diffusion of substances from the cytoplasm to the karyolymph has made the two somewhat similar, which results in the disappearance of the interface or membrane that separates the nucleus and cytoplasm. KUWADA (**3**) found dehydration and hydration to be factors in chromosome coiling and uncoiling, and he suggests that hydration and dehydration phenomena may be related to many problems concerning mitosis and meiosis.

Doubtless the enlargement of first prophase meiotic nuclei influences chromosome behavior by increasing the space in which they are limited and by decreasing the viscosity of the karyolymph within which they move.

The relationships between length of chromosomes in different stages of mitosis and meiosis have been determined by SAX and SAX (**6**). They found the prophase to metaphase proportions in root tip cells to average about 3:1. The proportional length of prophase to metaphase chromosomes in meiotic cells was about 9:1. A most important point they brought out was that prophase meiotic chromosomes are from 1.4 to 2.4 times longer than prophase mitotic chromosomes. They indicated that greater length of meiotic chromosomes is the result of their being uncoiled almost completely, which permits intimate gene by gene chromosome pairing. This is impossible in somatic chromosomes, for they are always coiled. No doubt the great increase in size of first prophase meiotic nuclei is important in allowing chromosomes to expand to their full length by completely uncoiling, which is hardly possible in smaller nuclei.

Large meiotic prophase nuclei can possibly have another function, in that the greater size permits chromosomes to separate to such distances that the mutual repulsion of non-homologous chromosomes is no longer effective in keeping chromosomes in place; that is, it allows chromosomes some freedom to move independently of one

another. In smaller mitotic nuclei the repulsion of non-homologous chromosomes probably tends to keep chromosomes in the same relative position.

It is suggested (**6**) that there is an attraction between homologous chromosomes at all times, as indicated by occasional somatic pairing. Other evidence of a mutual attraction between homologous somatic chromosomes is paired salivary chromosomes of *Drosophila*. They are in an enlarged nucleus (**2**), and they remain organized as definite prophase chromosomes for a long time. It seems possible that homologous chromosomes would always associate if the karyolymph were sufficiently fluid, the prophase of sufficiently long duration, and the nucleus sufficiently large.

As has been pointed out, another difference between meiosis and mitosis is in the time required for each. The mitotic cycle has been reported to require from 30 to 340 minutes. TAYLOR (**8**) found in *Gasteria* that the resting period after the last premeiotic division was a long one, in which there was considerable increase in size. The total time necessary for meiosis in *Tradescantia* is estimated by SAX and EDMONDS (**7**) to be five or six days. Comprehensive data are unavailable regarding the comparative time required for mitosis and meiosis, but there can be little doubt that the prophase of meiosis is much longer than the prophase of mitosis.

It is seen from the preceding three points (great increase in prophase meiotic nuclear size, marked changes in viscosity of karyolymph, and comparatively great time required for meiosis) that chromosome behavior during meiosis may be more fully explained by attributing importance to other factors in addition to those considered in the current theories. In all divisions there seems to be an attraction between homologous prophase chromosomes, and the particular conditions existing in prophase of meiosis allow the chromosomes to move together and to satisfy the attraction. Perhaps it is entirely a matter of time in the prophase stage, size of nucleus, and viscosity of karyolymph that permit chromosomes to pair. Such a suggestion seems to fit most of the cytological observations, including observations that some chromosomes are split at leptonema stage, that some are paired in somatic cells, and that multiple-strand chromosomes pair in the salivary glands of *Drosophila*.

Summary

1. Prophase mitotic nuclei are 1.44 times greater in volume than resting mitotic nuclei, while prophase meiotic nuclei are 3.52 times greater in volume than resting meiotic nuclei; and meiotic prophase nuclei are 3.25 times greater in volume than mitotic prophase nuclei.

2. Apparently the increase in meiotic nuclear size is accompanied by a decrease in viscosity of karyolymph, which results in extremely liquid karyolymph at the zygonema stage.

3. The time required for meiosis is much longer than that required for mitosis.

4. A hypothesis is advanced that the increase in volume of meiotic prophase nuclei, the decrease in viscosity of the karyolymph, and the comparatively long duration of meiosis, operate harmoniously to allow intimate gene by gene association of homologous chromosomes in the first prophase of meiosis.

BIOLOGICAL LABORATORIES
HARVARD UNIVERSITY
CAMBRIDGE, MASSACHUSETTS

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NOTE OF AN UNUSUALLY TRANSMITTED LETHAL EFFECT IN X-RAYED SORGHUM

R. M. WHELDEN AND C. P. HASKINS

(WITH ONE FIGURE)

Introduction

Many references occur in the literature concerning gross somatic as well as genetic effects in plants treated with x-rays. Such pioneer work as that of LOPRIORI[1] on *Vallisneria*, indeed, was reported very shortly after the discovery of x-rays and the design of the first gas tubes, and the work which has been reported since that time has reached enormous proportions. So far as known to the writers, however, all gross somatic modifications observed in plants arising from x-rayed seeds have occurred in the first generation; that is, in the seedlings arising from the treated seeds. The observed effects in the generation of plants arising from those which have been modified have been due either to typical genic modifications or to deficiencies in endosperm or other deformities of the seeds of plants which were themselves deficient.

It is the purpose of this paper to report a case which is somewhat different, and to the best of our knowledge, unique, involving the uniform production of deformed and lethal plants arising from seeds borne by plants, themselves of normal growth and form, which had been x-rayed as seeds, when such seeds had been rayed beyond a given critical minimum dosage. In brief, the effect observed skipped a generation, and then produced results not at all dissimilar to those ordinarily observed in a first generation of overdosed seed.

Material and method of handling

The effect here described was observed accidentally in the course of a study of the behavior of a single-factor recessive gene for albinism under x-irradiation. The material used was a strain of sorghum developed by Dr. R. E. KARPER at the University of Texas, and

[1] Schaudin in Pflüger's Arch. **77**:31. 1899.

described by him some time ago.[2] Through his kindness our original stock was obtained from Texas in 1932, and has been bred extensively here since that time. It has been found of high uniformity, and to behave consistently as described by KARPER.

The original purpose of the study was an investigation of modifications of the normal green-white ratio in seedlings arising from heads of sorghums, the parent plants of which had been subjected to fairly heavy dosages of x-rays as seeds. Seeds were therefore x-rayed and planted, and only those for which the applied dosage was small enough to permit the seedling to survive under field conditions were saved. These were allowed to grow to maturity in the field, were bagged before flowering, so that all daughter seeds were selfed, and were harvested. An individual record was kept of each head, and the young seedlings were grown in the laboratory on agar in Erlenmeyer flasks. It was during this procedure that the effect was noticed. The original seedlings were of course both homozygous and heterozygous for green, the homozygous whites consistently dying when the seed endosperm was exhausted. At maturity of the parent plant, however, each head was tested, and only the seeds from heterozygous green-white plants were germinated. Heterozygous and homozygous green plants are indistinguishable morphologically, as described by KARPER.

Source of radiation

The source of radiation used was a Coolidge deep-therapy thick-walled tube, water-cooled and with tungsten target, operated at 200 kvp. and 30 ma., with Snook mechanical rectification, and delivering, at the point used, 120 röntgens per minute. The distance from the target to the seed was 50 cm. The seeds were mounted on a lead plate, resting on a wooden table in a lead-lined room, so that there was considerable backscatter. The shortest wave length theoretically obtainable at this voltage is about 0.07 Å., and it is probable that a considerable continuous distribution toward longer wave lengths was present. No filter was used. Use of the tube was made possible through the courtesy of the Research Laboratory and

[2] Genetics 16:291–308. 1931.

the Vacuum Tube Engineering Department of the General Electric Company at Schenectady.

Seeds were exposed dry, under the conditions described, for periods of 4, 8, 16, 32, 64, and 128 minutes, and planted in the open field. Seedlings from the group of longest exposure were uniformly malformed, feeble, stunted, and perished early in the field. The rest matured well. They were planted at an experimental farm on the grounds of the Schenectady plant of the General Electric Company and on the grounds of the Gray Herbarium of Harvard University in Cambridge, Massachusetts. All plants were bagged when the flowers were in young bud, "glassine" wax paper bags being used for this purpose. The seeds were harvested at maturity, those from each head being placed in a separate container labeled with the head number and exposure time of the parent seed.

The young seeds from heterozygous plants were germinated as described earlier, both sterile pure agar and agar containing nutrient medium being used. The latter was made up with Hoagland's solution and 20 per cent agar, and was adjusted to a pH of 6.5 to 7.0. Initial growth was independent of the constitution of the agar. The flasks used were of the wide-mouthed type, of one liter capacity.

Results

Seeds from plants rayed as seeds up to 32 minutes behaved perfectly normally. The progeny of the 64 minute group, however, were malformed and defective, although the parent stock had been perfectly vigorous.

The shoots of seedlings of the 64 minute group behaved very uniformly. After reaching a few millimeters in length they became brown. Almost no development of internodes occurred, and the leaves were short, stubby, and tended to curl backward markedly, the latter effect being especially noticeable in the homozygous white seedlings (fig. 1). Otherwise there was no distinction in behavior between white and green seedlings, both browning and dying long before they had reached the heights attained by controls of either group. Control plants grew to 150–400 mm. shoot length, with an abundant root system. Early curling and browning were likewise absent in the controls of both green and white plants.

The roots showed somewhat greater variability in size and form within the 64 minute group. In most cases they were stunted in

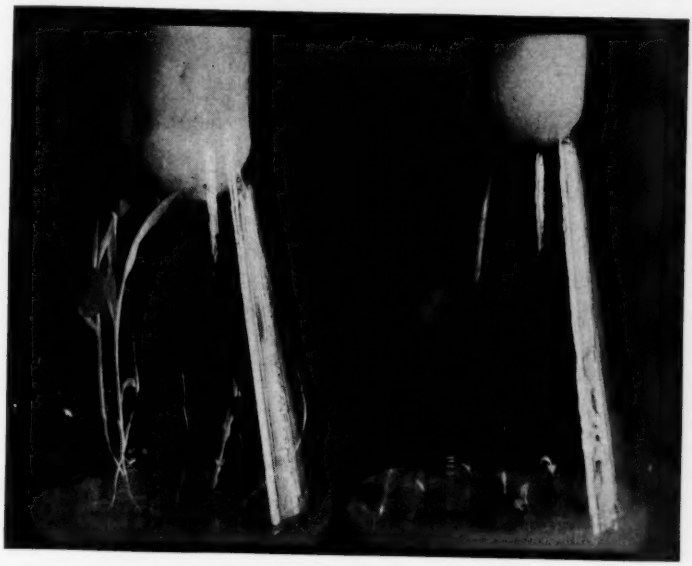

FIG. 1

TABLE 1

HEAD NUMBER	NO. PLANTS COUNTED	AVERAGE LENGTH OF SHOOT (MM.)	AVERAGE LENGTH OF ROOT (MM.)
4–64	49	11.8	28.9
8–64	134	12.9	39.7
11–64	20	11.6	19.9
12–64	113	14.9	35.2
14–64	74	12.1	19.0
17–64	123	11.3	21.1
Total	513	Ave. 12.6	Ave. 29.5

length and were very sparingly branched. Occasionally long root systems occurred, but they were composed of very slender rootlets. No correlation could be found between size of root and of shoot.

Microtome sections showed little difference between plants of the 64 minute group and controls, although there was a suggestion of abnormality of conducting tissue from a very early stage of development.

In all, more than 2000 plants were used in testing the effect described, and of these but two failed to show it. It seems possible that they represented seeds accidentally introduced. The data for 513 individuals are shown in table 1.

The results are clearly indicative of the uniform occurrence of what are usually considered somatic effects in the unrayed progeny of plants, themselves visibly unaffected, experiencing somewhat high dosages as seeds. Further work is in progress, and it is hoped to elucidate the nature of the phenomenon.

BIOLOGICAL LABORATORIES
HARVARD UNIVERSITY

HASKINS LABORATORIES
UNION COLLEGE
SCHENECTADY, NEW YORK

FATIGUE, SUMMATION, AND DAILY VARIATION OF IRRITABILITY IN SENSITIVE STIGMAS

HARRY J. FULLER AND JOHN H. HANLEY

(WITH TWO FIGURES)

Introduction

Although numerous observations have been made and numerous experiments performed upon those members of the Scrophulariaceae, Bignoniaceae, Martyniaceae, and Lentibulariaceae whose stigmas exhibit turgor responses to contact and other types of stimulation, the phenomena of fatigue, summation of stimulation, and daily variation in sensitivity have been neglected. This paper presents the results of a series of experiments with reference to these subjects upon the sensitive stigmas of *Torenia fournieri*, *Catalpa speciosa*, *Tecoma radicans*, and *Mimulus luteus*. In so far as the writers have been able to determine, only one paper upon any of these subjects has thus far appeared, that of BROWN (1) who described fatigue in the stigmas of *Martynia*. The papers of NEWCOMBE (5), BURCK (2), and MACBRIDE (4) present inclusive reviews of the earlier observations upon sensitive stigmas.

Fatigue

The stigmas of flowers of the species mentioned were stroked with a sliver of wood, and after closing of the stigmas, the time necessary for full reopening was determined. As soon as the stigmas had opened to the maximum, they were stimulated again and the time necessary for complete recovery of the unstimulated position was again noted. This repeated stimulation was continued until the stigmas remained closed, or until a recovery period of one hour was attained. The results of these experiments are presented in table 1.

It is shown in the table that with each successive stimulation the period necessary for complete recovery becomes longer. In *Torenia* and in *Tecoma* and *Catalpa* no stigmas reopened during an hour's time after the third and fourth stimulations, respectively, whereas in *Mimulus* the stigmas in fourteen of the fifteen flowers reopened after

the fifth stimulation, but did not reopen during an hour after this stimulation.

Daily variation in sensitivity of stigmas

Stigmas of *Torenia* and *Mimulus* were stimulated by the induced current, with one electrode from the induction coil in contact with the contact-insensitive outer surface of a stigma lobe and the other

TABLE 1

RECOVERY PERIODS IN MINUTES FOLLOWING STIMULATION
AVERAGES OF DETERMINATIONS UPON FIFTEEN
FLOWERS OF EACH SPECIES

STIMULATION	TORENIA	MIMULUS	TECOMA	CATALPA
First	34	14	19	23
Second	43	17	24	29
Third	58	21	29	31
Fourth	.....	37	38	45
Fifth	.....	55	.....	.....

FIG. 1.—Unstimulated and stimulated stigmas of *Torenia fournieri*

electrode against the style 5 mm. below the stigma (fig. 1). The stigma was stimulated at 3-hour intervals during the day and night. The minimal stimulus required for closure at each time was taken as the index of sensitivity. Ten stigmas of each species were treated in this manner. The results are shown in figure 2, in which the value 100 represents the maximum sensitivity value, that of *Mimulus* at 3 P.M. The graph shows that sensitivity in both species rises during

the forenoon and early afternoon to a maximum between 3 and 6 P.M., after which it gradually decreases during the night to reach a minimum between 3 and 6 A.M. This variation in sensitivity corresponds roughly with that of the turgor response of *Mimosa* reported by BURGE, WICKWIRE, and FULLER (3), except that in the stigmatic responses the range of sensitivity during the 24-hour period is considerably less than that of *Mimosa*. The slight differences in some values are possibly within the experimental error, but the

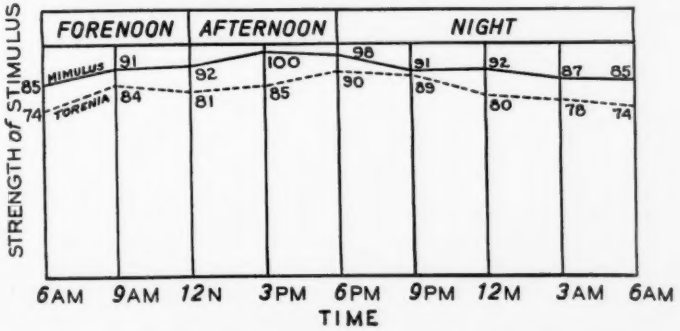

FIG. 2.—Daily variation in sensitivity of stigmas of *Mimulus* and *Torenia*

coincidence of the curves for the two species seems to indicate that sensitivity is at least slightly higher during the sunlit hours of the day than it is at night.

The graph shows also that the stigmas of *Mimulus* are slightly more sensitive than are those of *Torenia*.

Summation of stimulation

By repeated application of the induced current in subliminal quantities, summation of stimulation with resultant closure of *Torenia* and *Mimulus* stigmas was obtained. Ten stigmas of *Torenia* and eight of *Mimulus* were treated. In all cases subliminal stimulations of less than 70 per cent of the threshold value failed to produce summation, and intervals of longer than fifteen seconds between stimulations likewise failed to produce summation. The number of applications of subliminal stimuli necessary to induce the response varied with different flowers from three to seven.

Summary

1. Fatigue may be demonstrated in the stigmas of *Catalpa speciosa*, *Tecoma radicans*, *Mimulus luteus*, and *Torenia fournieri*.

2. The stigmas of *Mimulus luteus* and of *Torenia fournieri* are somewhat more sensitive to stimulation during the day than during the night.

3. The stigmas of these species respond as a result of a summation of stimulations of not less than 70 per cent of the threshold value presented at intervals of not more than fifteen seconds.

DEPARTMENT OF BOTANY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

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HISTOLOGICAL RESPONSES OF THREE SPECIES OF LILIUM TO INDOLEACETIC ACID[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 491

J. M. BEAL

(WITH SIXTEEN FIGURES)

The gross responses of *Lilium philippinense formosanum* and *L. harrisii* to applications of indoleacetic acid in lanolin to the cut surfaces of decapitated stems were described in a previous paper (1). The former species responded by the production of adventitious roots from approximately the surface of application to as far down as 2.5 cm. from it, while the latter produced buds in the axils of the two or three leaves nearest the surface of application, with relatively few, and in most stems no, adventitious roots. The buds also have since been shown to be strictly adventitious in origin.

In the first series tested some of the plants of *L. philippinense formosanum* had nearly mature floral buds at the time the experiments were begun. In a few cases the pedicels were cut off about 1 cm. below the receptacles and treated with the lanolin mixture. These responded in essentially the same time interval and in much the same way as did the treated stems.

A few of the floral buds were permitted to flower. The lanolin mixture was smeared over the unpollinated stigmas of some of the flowers, while in others the ovary was cut off about 5 mm. below the base of the style and the cut surface was then covered with the mixture. In both cases the ovaries enlarged somewhat, and remained green and succulent for an appreciably longer time than did pistils which were untreated and unpollinated. None of them continued to develop parthenocarpically, as was reported by GARDNER and MARTH (3) for the holly and strawberry, and by GUSTAFSON (5, 6) for various other plants. After a period of 10 to 15 days they began to shrivel and shortly afterward yellowed and died. Neither roots,

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

such as KRAUS, BROWN, and HAMNER (10) described on treated bean pods, nor buds were stimulated to develop on any of the treated ovaries.

Following the first experiments, which were carried out in the autumn of 1936 and the winter of 1937, it seemed advisable to test also *L. longiflorum* Thunb., a species to which *L. harrisii* Carr. is sometimes assigned as var. *eximium* Nichols. It was surprising to find that it responded in almost precisely the same manner as had *L. philippinense formosanum* in the production of adventitious roots, and that it produced no adventitious buds such as were developed by *L. harrisii*, to which it is so much more closely related. The three species used belong to the Eulirion section of the genus *Lilium*.

Approximately one hundred additional plants of *L. harrisii*, fifty of *L. philippinense formosanum*, and forty of *L. longiflorum* were used in a second series of tests made in the spring and summer of 1937. Bulbs of the respective species were potted in garden soil in 6 inch clay pots and kept under average greenhouse conditions. When the stems had reached a height of 6 to 8 inches they were decapitated just below the apical bud, which consists of a much shortened axis and a number of leaves of varying sizes and degrees of maturity. After decapitation, a single application of a 3 per cent mixture of indoleacetic acid in lanolin was spread as evenly as possible over the entire cut surface. Decapitated untreated stems were left as checks in each species and in each series.

Material for histological studies was collected at 24 hour intervals following treatment, fixed in Navashin's solution, and run up according to the butyl-alcohol paraffin method. Sections were cut at 12 μ and stained in a modified triple stain.

Because of the markedly different responses of *L. philippinense formosanum* and *L. longiflorum* on the one hand and of *L. harrisii* on the other to the lanolin mixture, the two types of responses are discussed separately.

L. philippinense formosanum and L. longiflorum

GROSS RESPONSES TO TREATMENT

Following decapitation and application of the lanolin mixture, only slight gross response is evident for several days. At the end of

about 48 hours a faint yellowing of the tissues adjacent to the surface of application becomes evident. During the third or fourth day the yellowing becomes more pronounced and may extend down the stem for a distance of 2 to 3 mm. The stems usually show a slight amount of swelling for a distance of 0.5 to 1 cm. below the cut surface, and the swelling may continue slowly for a period of several days, but never becomes very marked. After 10 days to 3 weeks small protuberances begin to appear from approximately the surface of application to as far down as 2.5 cm. below. The protuberances result from developing adventitious roots. In a few instances roots push up through the surface of application, but these are relatively rare as compared with the numbers which develop below this surface and which push out through the epidermis of the stem.

A small amount of callus may also develop at the surface of application in contact with the lanolin mixture, but it is usually scanty and in most examples is entirely wanting. Both callus formation and swelling of the treated stems are much less pronounced than in the bean (**10**) or as reported by HARRISON (**7**) in *Iresine*.

When the treated plants are allowed to grow for a period of 5 to 6 weeks under ordinary greenhouse conditions, the roots may push out to a length of 2 cm. or more (fig. 1*B*). If placed under humid conditions they elongate more rapidly, become much longer, and may remain alive for many weeks at least. Under either condition they often show a green color owing to the development of chlorophyll.

The check plants (fig. 1*A*) usually develop no callus on the cut surface, no swelling below it, nor do they in any case produce adventitious roots at or near the cut surface.

The treated pedicels respond in essentially the same manner as do the treated vegetative stems. They show the same type of yellowing and about the same degree of swelling, and after about the same time interval protuberances similar to those on the stems become evident.

HISTOLOGICAL DETAILS

The vascular bundles in the stem of *Lilium* are scattered irregularly throughout the ground meristem or fundamental parenchyma, and are of the closed collateral type. The provascular strands become recognizable a short distance back from the stem tip, and con-

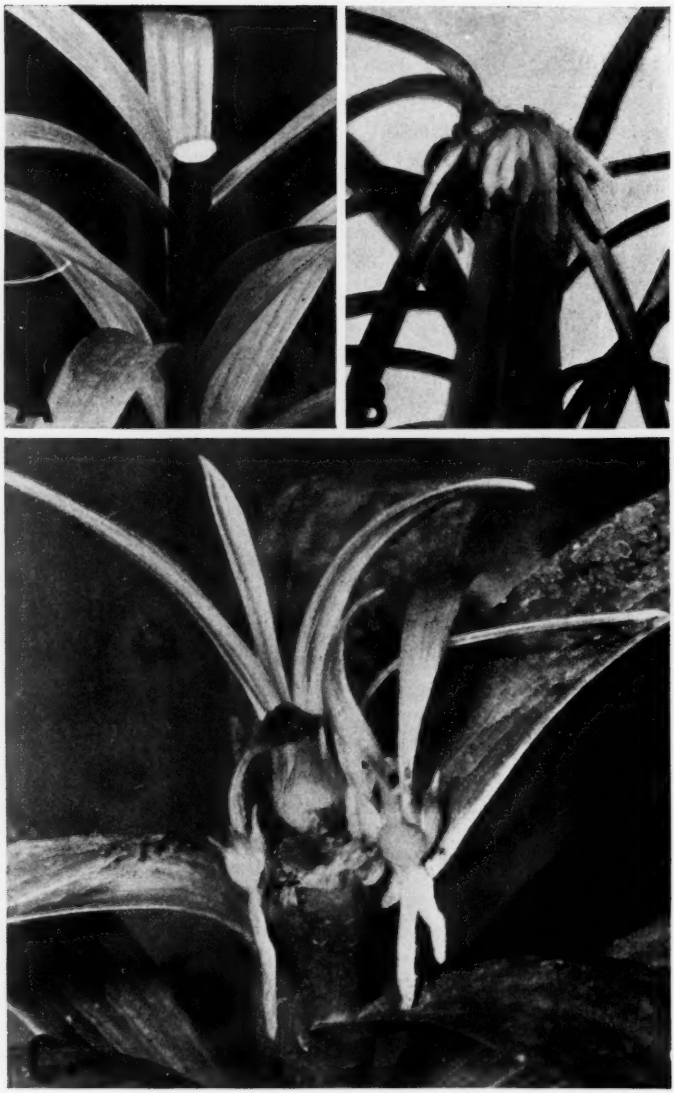

FIG. 1.—*A:* decapitated untreated stem of *L. harrisii* 12 weeks after excision (×⅔). *B:* decapitated treated stem of *L. philippinense formosanum* 8 weeks after treatment, showing crown of adventitious roots (natural size). *C:* decapitated treated stem of *L. harrisii* 12 weeks after treatment; three buds (bulbils) have developed, two in axil of uppermost leaf and one in axil of second leaf (×2½). Roots have developed from bulbils but not from stem.

sist of cells which are smaller and more uniform in size and with a denser cytoplasmic content than those of the fundamental parenchyma, because the cells of the latter enlarge more rapidly and differentiate earlier. Protophloem elements are differentiated in the outer third of the provascular strands and apparently become functional before protoxylem becomes evident. There is no indication of a distinguishable endodermis or pericycle associated either with the strands or with the bundles into which they develop. Whether or not a cambium is present is a matter of interpretation. In the larger and older bundles, in which a considerable amount of phloem and xylem has been formed, there is often present a group of radially flattened and tangentially elongated cells which may divide and from their derivatives additional phloem and xylem elements develop. The xylem thus derived shows annular or spiral thickenings, however, just as does the first formed metaxylem. Critical examination of older stems in longitudinal section has failed to show the presence of pitted vessels or tracheids in any bundle observed.

At the time of application of the lanolin mixture the region of the stem at the level of decapitation has the main regions well defined, although it is obvious that most of the cells have not yet reached maturity. The epidermal and outer cortical cells have comparatively dense cytoplasm and prominent nuclei (fig. 2*A*). Chloroplasts are present in many of the outer cortical cells. The more centrally placed vascular bundles show a number of differentiated primary phloem elements, a few protoxylem elements, and the beginning of differentiation of metaxylem. A few of the peripheral bundles are in a similar stage of development, but many of them show little differentiation other than the beginning of protophloem formation, and are still in essentially the provascular stage. The protophloem seems always to begin differentiation in advance of the protoxylem.

Twenty-four hours after application of the lanolin mixture little recognizable change is apparent except perhaps a slight increase in size of the cells of the epidermis, outer cortex, and fundamental parenchyma. As these cells enlarge their vacuoles become more pronounced, and in general their nuclei less prominent. The first pronounced responses resulting from the application of the mixture be-

come observable in stems collected 3 days after treatment (fig. 2*B*). Relatively little additional development has occurred in the vascular bundles, but some of the fundamental parenchyma cells immediately adjacent to them show an increase in the amount and density of their cytoplasm and more intensely staining nuclei. Four days after treatment the changes are even more marked (fig. 3*A*). A greater number of the cells of the fundamental parenchyma show an increased amount of cytoplasm and certain of them have begun division. A detailed view of one vascular bundle is shown in figure 3*B*. In this figure it is obvious that the responses to application of the indoleacetic acid are limited almost entirely to the cells of the fundamental parenchyma, and that few if any of the cells of the vascular strands are involved at this stage of development. In the later stages of root development, cells of the vascular bundles which have remained undifferentiated become transformed into vascular elements which serve to connect the newly formed elements of the adventitious roots to those of the original bundles of the stem. Following nuclear division a cell plate is formed immediately and wall formation quickly ensues. Thus apparently a multinucleate condition is never attained.

A moderate amount of variation in the rate of response to treatment occurs in different stems; the more highly vegetative the stem at the time of application of the mixture, the more rapid its response. Some of the stems, 5 or even 6 days after treatment, will show about the same condition as that in figure 3. Some of them develop many more roots than others. In general, however, any given lot of plants subjected to the same type of treatment exhibits a high degree of uniformity in behavior.

Following the condition just described (fig. 3), repeated mitotic divisions occur during the next few days, resulting in the production of clearly defined groups of highly meristematic cells which are usually localized in a region adjacent and centrifugal to the phloem, but which at times may almost completely surround the bundles (fig. 4*A*). This figure is from a stem collected 9 days after treatment. Infrequently the groups may lie largely at one side of the bundles. By continued divisions, and differentiation of their derivatives, these groups of cells give rise to cells which will later develop into the ad-

ventitious roots. Figure 4*B* shows the detailed structure of the young root at the right in figure 4*A*. The outlines of the original parental cell walls are often clearly recognizable even after repeated divisions have occurred within them. This is even more strikingly demonstrated in figure 5, taken from a transverse section of a stem of *L. longiflorum* 16 days after treatment, in which the sequence of events parallels almost exactly that in *L. philippinense formosanum*. The original parental cell walls persist in recognizable form for a period of several days, but sooner or later are no longer distinguishable, perhaps as a result of stretching and consequent thinning, caused by growth of the derivatives formed within.

A more advanced stage in root development is shown in figure 6. The general relation of the adventitious roots to the vascular bundles, as well as their origin from the fundamental parenchyma, is here clearly defined. So general are the mitotic divisions throughout this group of highly meristematic cells that if histogens are developed at these early stages they are not clearly indicated. As the cells continue proliferating and the whole meristematic mass increases in volume, it encroaches laterally and centrifugally upon the fundamental parenchyma which has not responded visibly to the treatment, crushing and then digesting its way through these cells and those of the outer cortex, and finally rupturing the epidermis. Later stages of root formation are shown in longitudinal sections (fig. 7*A*, *B*). These are from a stem 13 days after treatment, and show well defined roots. Evidence of an apical histogen is discernible in figure 7*B*, and cell elongation in the central core of cells foreshadows the beginning of vascular differentiation.

Following the general blocking out of the root, xylem elements are differentiated near the base and periphery of the inner core of cells. The first xylem differentiated appears always to be tracheids, which connect the vascular systems of root and stem. Simultaneously phloem is differentiated in the same general region but on alternate radii, and it also forms a continuous system between root and stem. New tissues are added apically, and as growth continues the young root pushes out through the covering layers of the outer cortex (fig. 8). The main regions are clearly distinguishable, and through continued growth and under favorable conditions the roots quickly

emerge from the stem and mature in much the same way as do secondary roots. The induced roots appear normal in all respects.

The treated pedicels, while generally responding to the treatment like the stems of *L. philippinense formosanum*, show one rather marked difference. Nearly all, and in some cases all, of the vascular bundles are associated with the production of roots (fig. 9). These are developed in all respects in the same way as are those from the stems of the same species.

Lilium harrisii

Gross responses to treatment

Yellowing and slight swelling of the stems immediately below the surface of application occur in this species much as in the two previously discussed, but there is a greater range of variation in response in *L. harrisii*. A larger amount of callus may occasionally develop over the surface of application, and in some stems may even develop in the axils of the upper one or two leaves. A few of the treated plants develop adventitious roots, but these almost invariably come from the callus tissue rather than from the fundamental parenchyma about the vascular bundles, as occurs in the other two species described. Large tumors have not been produced by any of the treated stems.

By far the most striking change is the development of the buds, not visible at the time of treatment, in the axils of the upper two or three leaves (fig. 1*C*). In a very few cases buds develop from the callus tissue on the cut surface of the stem, but these cases may possibly be associated with cells in the leaf axil where the plane of cutting the stem passed through or partly through this region. In the great majority of cases observed the buds arise directly in the leaf axils without the formation of callus and upon stems from which no adventitious roots arise. The number of buds developed per plant varies from one to as many as four. In one instance three were observed in a single leaf axil, but one or two is the usual number.

The buds arise near the side of the leaf attachment as frequently as in the central or strictly axillary position. When two are present in an axil, one being strictly axillary and the other accessory in posi-

tion, they may be equal in size or either may outgrow the other. Apparently the one first formed will grow the more rapidly.

Two to 3 weeks after becoming evident, the buds begin to assume a bulbous form. At this time, and subsequently, they resemble closely the bulbils which form naturally near the bases of the untreated plants of this species. Figure 1*C* shows a plant about 3 months after treatment, which has developed four bulbils. The largest of these has leaves approximately 1½ inches in length, and all the bulbils have developed adventitious roots at their bases. Bulbils which have developed to the size of the largest one on this plant can be removed from the parent plant and grown independently in sand or soil.

None of the check plants produced either buds or roots over a period of about 4 months (fig. 1*A*), after which time the stems began to die.

Histological details

The structure of the normal stem of *L. harrisii* closely parallels that of the other two species. Both longitudinal and transverse sections have been cut, but since the clearest details of bud development have been shown by the longitudinal sections, these have been used exclusively for the photomicrographs of this species.

A median longitudinal section from a decapitated but untreated stem, typical of the stage of development at the level and time of application of the mixture, is shown in figure 10*A*. It is obvious that most of its cells have not reached maturity, although relatively few additional cell divisions would probably occur in this region under the usual conditions of growth. The cells contain comparatively large vacuoles with a rather scanty amount of cytoplasm surrounding them. The cell walls are thin.

Twenty-four hours after application of the mixture definite changes are observable (fig. 10*B*). The epidermal cells immediately above the leaf axil have enlarged, chiefly in the radial plane, while the outer cortical cells opposite them on the stem side as well as below the axil have also enlarged. An increase in density of their cytoplasm is evident, together with larger and more deeply staining nuclei. The cell walls throughout the entire section appear somewhat

thicker. The first observable changes in the treated stems are represented by this group of cells, which marks the general region of initial response at which the induced buds will appear later.

Little additional change, other than continued enlargement of cells in the region of the leaf axils and increase in the amount and density of their cytoplasm, is noticeable at the end of 3 days following application of the mixture (fig. 10*C*). A few nuclei show early prophase stages in mitosis, but as yet none has divided. Four days after treatment, some cells of both the epidermis and outer cortex have undergone division; although all the cells in this portion of the stem have continued enlargement, only those in the immediate vicinity of the leaf axil have started dividing (fig. 10*D*).

At the end of 6 days following treatment a larger number of the outer cortical cells have undergone division, and in many of them their derivatives in turn have divided (fig. 11*A*). The comparatively thick walls of the parental cells in this figure, as well as in the next, stand out conspicuously in contrast to the thin and scarcely distinguishable ones between the daughter nuclei. Just as in the other two species, they usually remain clearly evident for a period of several days, and may be recognized up to the eleventh or twelfth day following treatment; finally they are no longer discernible.

Subsequent changes proceed fairly rapidly. A stem collected 7 days after treatment shows a larger area of meristematic activity (fig. 11*B*). As a result of repeated divisions and subsequent growth of the derivatives, a hump of tissue has developed in the axil. The epidermal cells show increased radial elongation, but comparatively few divisions have occurred as yet in its cells. Eight days after treatment more of the epidermal cells have divided and the hump has become more pronounced (fig. 12*A*). Through further divisions and enlargement of the derivatives the condition shown in figure 12*B* is reached in about 3 days more, or 11 days after treatment. In the more actively growing portion of the embryonic bud it is no longer possible to distinguish the outlines of the original parental cell walls, although they are still visible in the center of the figure where growth has proceeded more slowly. A later stage in development is shown in figure 13, which is from a stem 14 days after treatment. The hump of cells seen in figure 12*A* and *B* has differentiated into an axillary bud

consisting of a growing point and two leaf primordia. The beginning of vascular differentiation is evident from a point near the base of the bud for a distance downward in the tissue of the stem. Provascular strands appear to be initiated a short distance back from the growing point of the buds, in essentially the same fashion as in the ordinary vegetative stems of *Lilium*, and in the same scattered arrangement. Differentiation of primary phloem and xylem proceeds in the same way.

Two more figures are shown to illustrate later stages in bud development. Both are from a stem 39 days after treatment, but figure 14 is from a somewhat less advanced stage of development than that of figure 15. Both show considerable evidence of vascularization, and from figure 15 it is only a short step to the formation of a bulbil such as those illustrated in figure 1*C*.

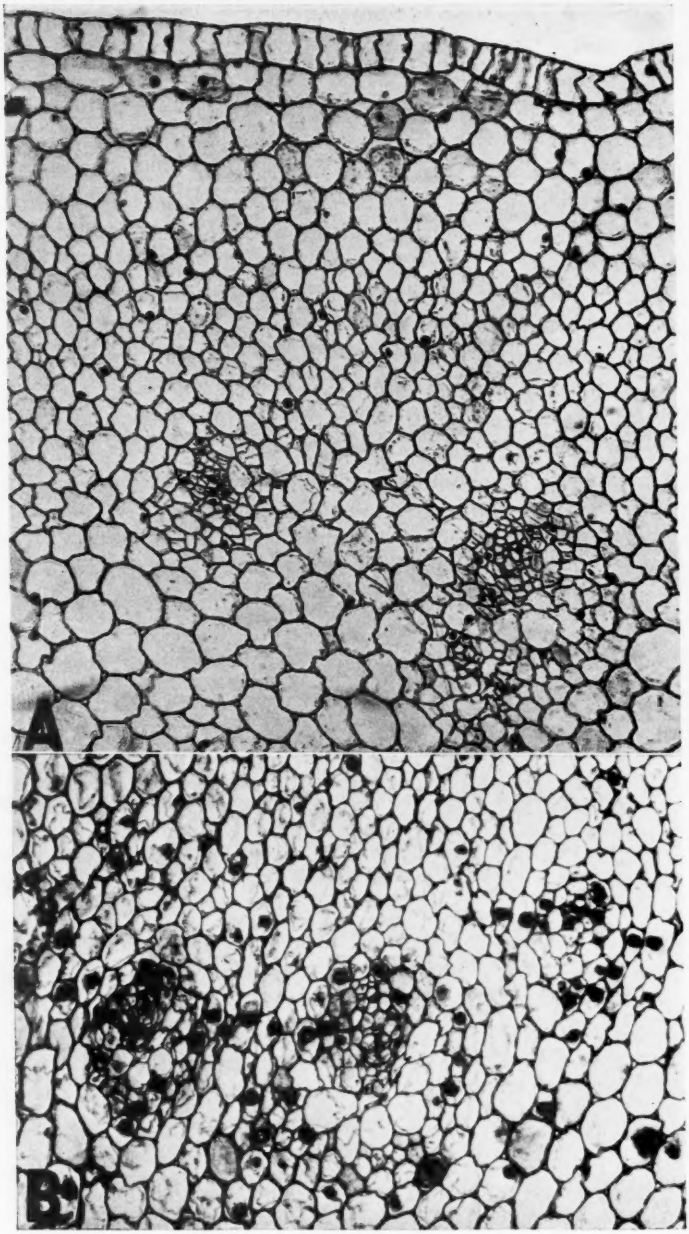

FIG. 2.—*L. philippinense formosanum. A:* transection about 2 mm. below treated surface at time of treatment; cells contain large vacuoles and inconspicuous nuclei. *B:* 3 days after treatment; fundamental parenchyma about vascular bundles shows denser cytoplasm and more deeply staining nuclei.

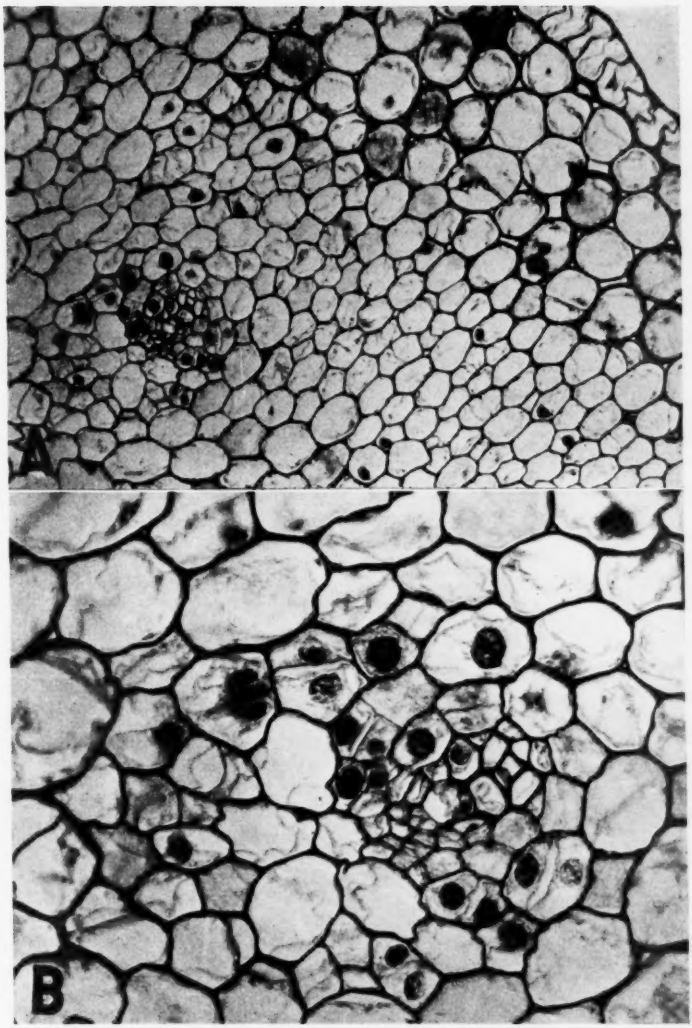

FIG. 3.—*L. philippinense formosanum* 4 days after treatment, about 4 mm. below treated surface. *A:* beginning of meristematic activity in fundamental parenchyma. *B:* details of same bundle enlarged. Phloem differentiated; primary xylem cells centripetal to those enlarging. Several fundamental parenchyma cells divided; cell walls separate their daughter nuclei. One cell shows cell plate formation.

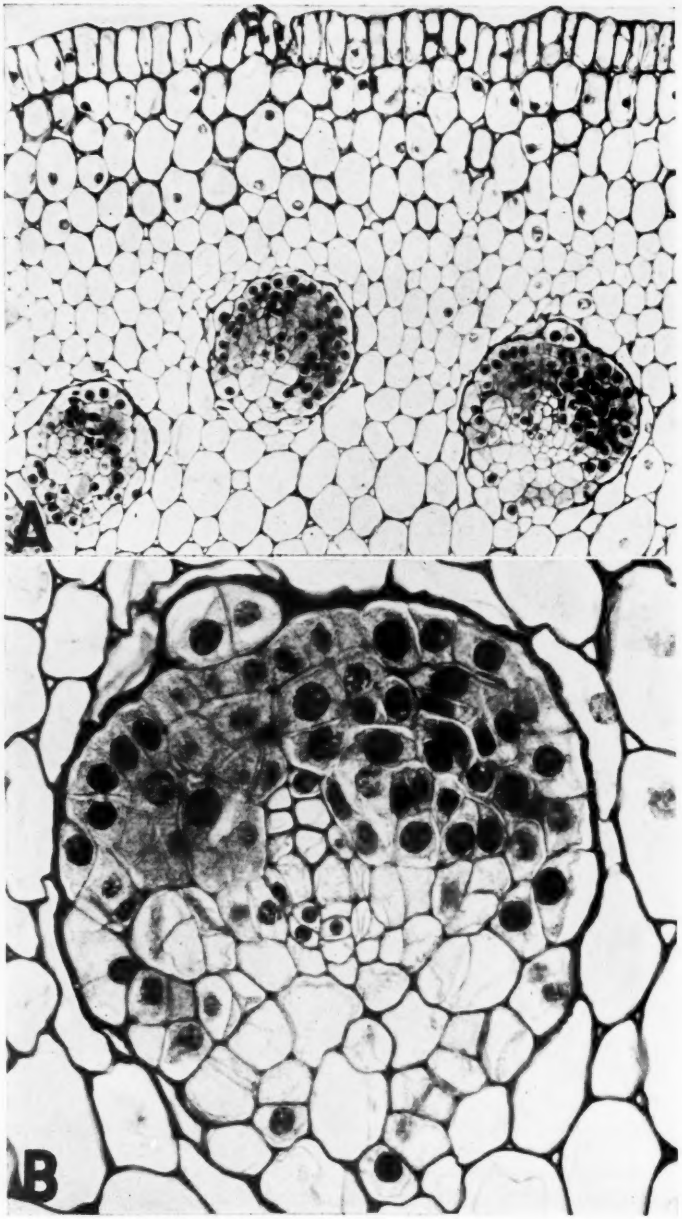

FIG. 4.—*L. philippinense formosanum* 9 days after treatment, section about 4 mm. below treated surface. *A:* marked increase of meristematic activity in fundamental parenchyma; xylem not fully matured. *B:* enlarged view of young root at right in *A*.

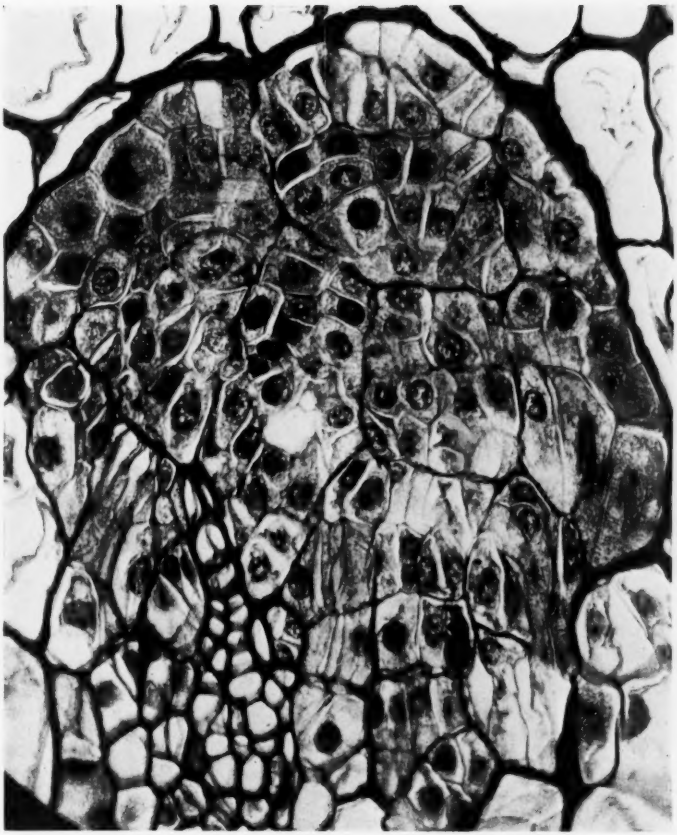

FIG. 5.—*L. longiflorum*, transection of stem 16 days after treatment, about 3 mm. below treated surface. Young root slightly more advanced than in fig. 4, showing original parental cell walls about their numerous derivatives. Cells of fundamental parenchyma which have not divided are being crushed.

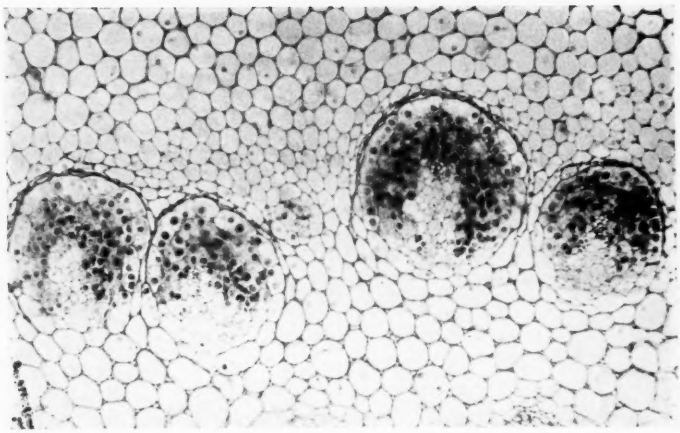

FIG. 6.—*L. philippinense formosanum*, more advanced stages than fig. 4. Meristematic activity marked in four young roots; crushing of fundamental parenchyma about growing roots.

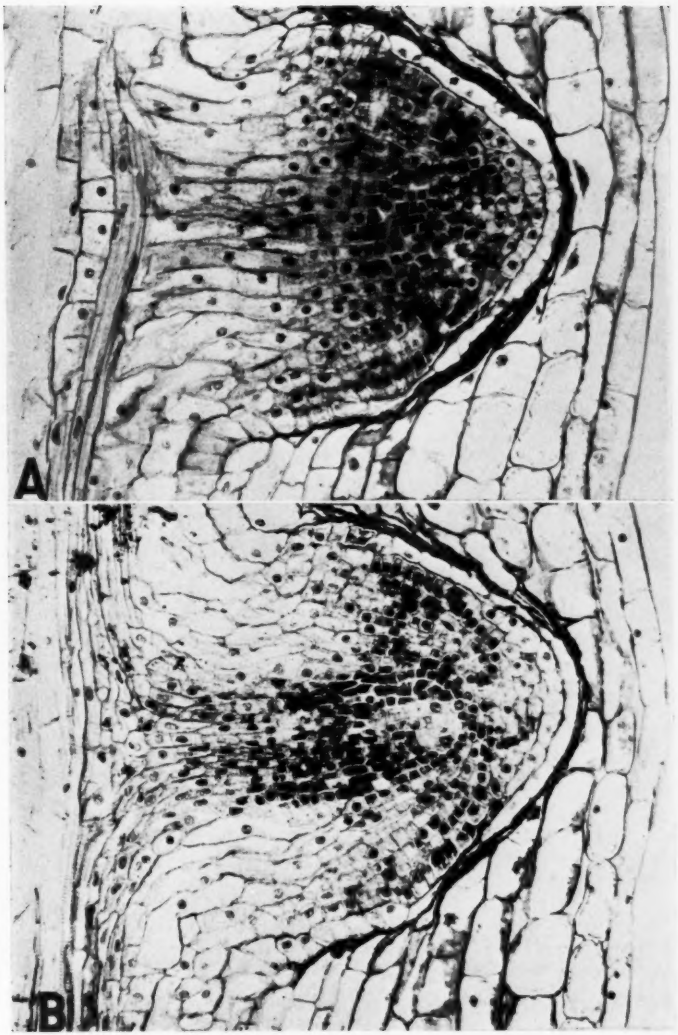

FIG. 7.—*L. philippinense formosanum*. *A:* 13 days after treatment, longitudinal section of stem and median longitudinal section of adventitious root. *B:* same age as *A*, more advanced. Apical histogen and regions of root differentiated.

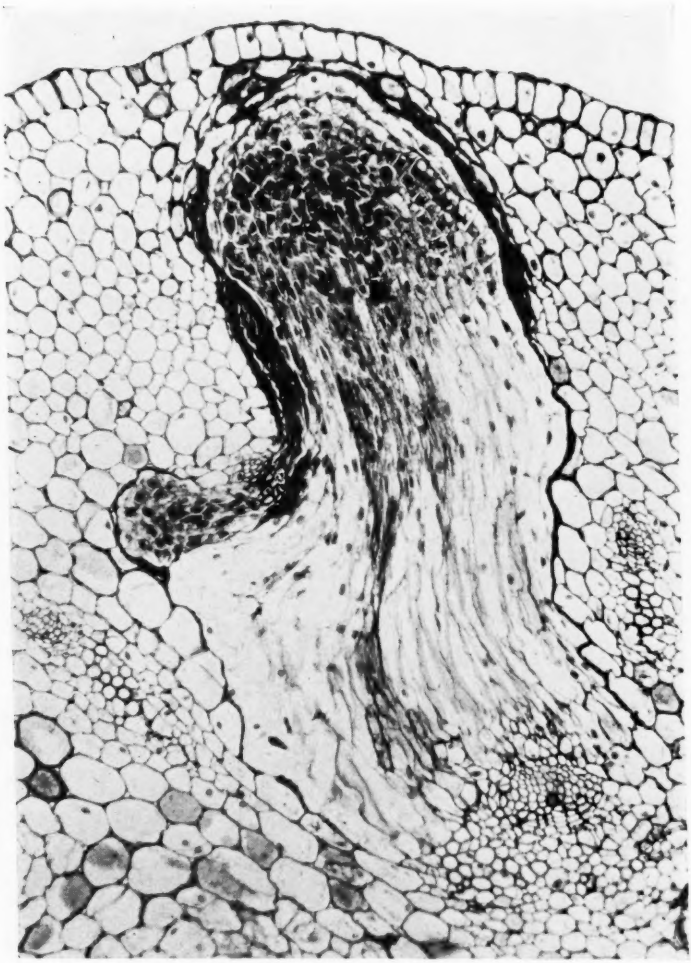

FIG. 8.—*L. philippinense formosanum* 21 days after treatment. Longitudinal section of stem and median longitudinal section of basal portion of adventitious root showing connection of xylem of root and of vascular bundle of stem.

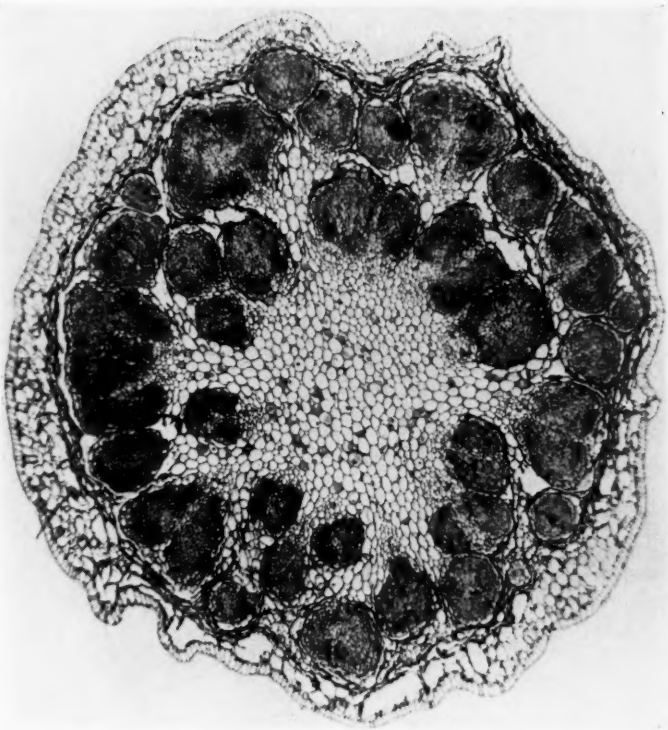

FIG. 9.—*L. philippinense formosanum*, transection of pedicel 21 days after treatment. Roots developing in association with all vascular bundles. Much crushing and disorganization of fundamental parenchyma and outer cortex.

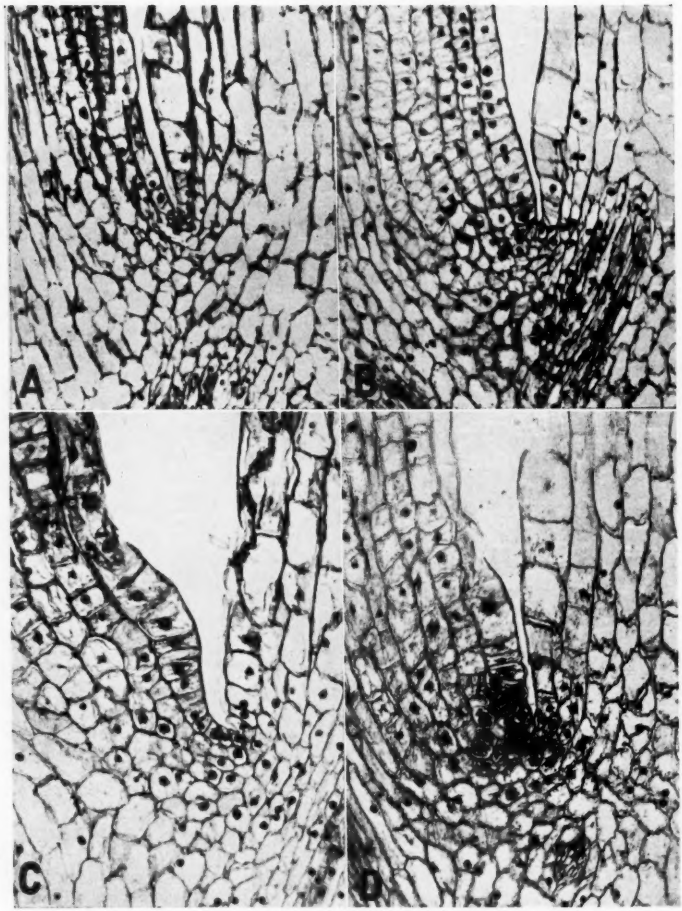

FIG. 10.—*L. harrisii*, longitudinal section through first leaf axil below surface of application. *A*: untreated check at time of treatment. *B:* 24 hours after treatment; epidermal cells above axil radially elongated; outer cortical cells near axil unchanged. *C:* 3 days after treatment; epidermal cells more elongated radially; outer cortical cells near axil with denser cytoplasm and prominent nuclei. *D:* 4 days after treatment; one epidermal cell is dividing and a few cortical cells have completed divisions.

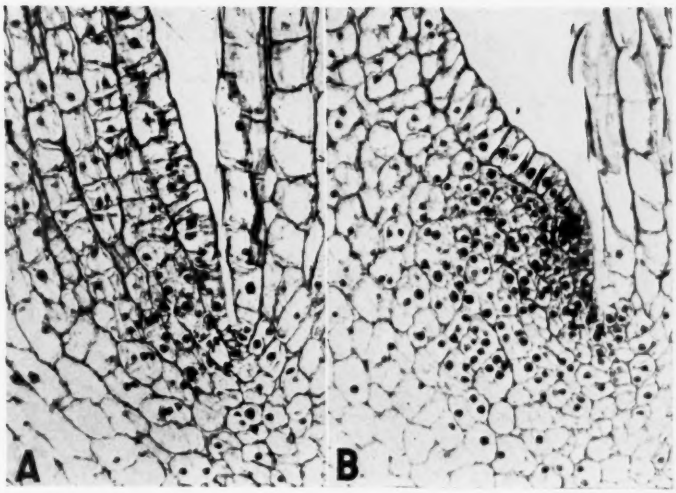

FIG. 11.—*L. harrisii*. *A:* 6 days after treatment. Many of outer cortical cells near axil divided, and their derivatives in some again divided. *B:* 7 days after treatment. Marked meristematic activity evident in fairly extensive area of cortical cells on stem side and below axil. Original parental cell walls evident, in some cases inclosing a number of derivative cells.

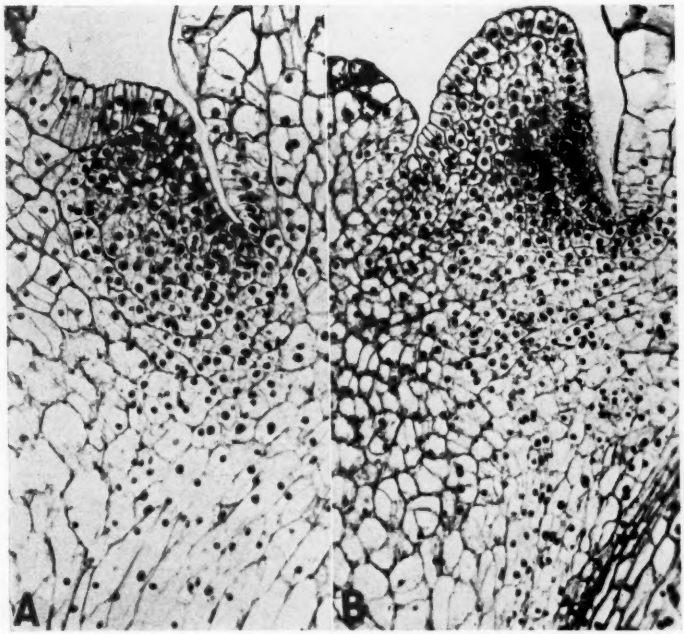

FIG. 12.—*L. harrisii*. *A:* 8 days after treatment; area of meristematic activity extended and growth of derivatives resulting in hump in axil. *B:* 11 days after treatment; hump much more pronounced. Original parental cell walls no longer recognizable in upper portion of embryonic bud, but still evident near center of figure.

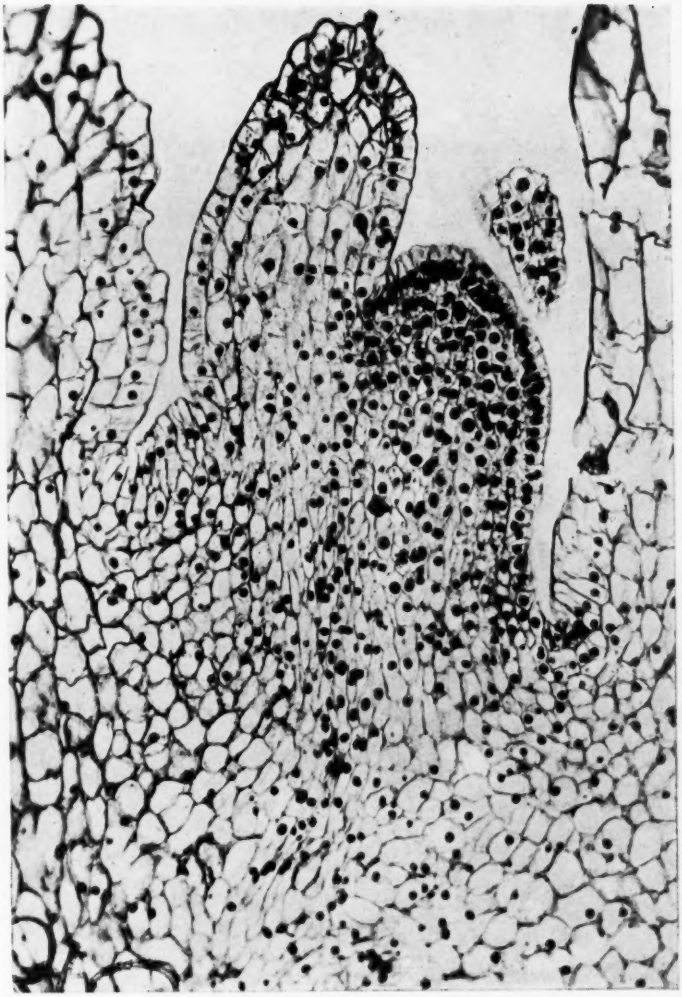

FIG. 13.—*L. harrisii* 14 days after treatment. Axillary hump, through continued proliferation and growth, developed into an axillary bud consisting of growing point and two embryonic leaves. Vascular differentiation beginning near center of figure and extending downward.

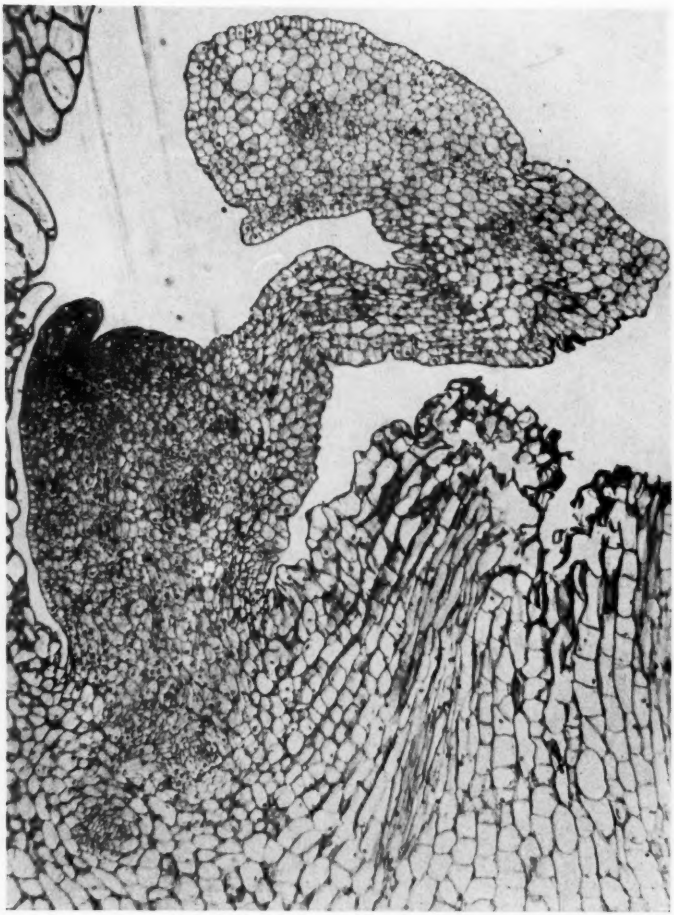

FIG. 14.—*L. harrisii* 39 days after treatment; more advanced stage than fig. 13

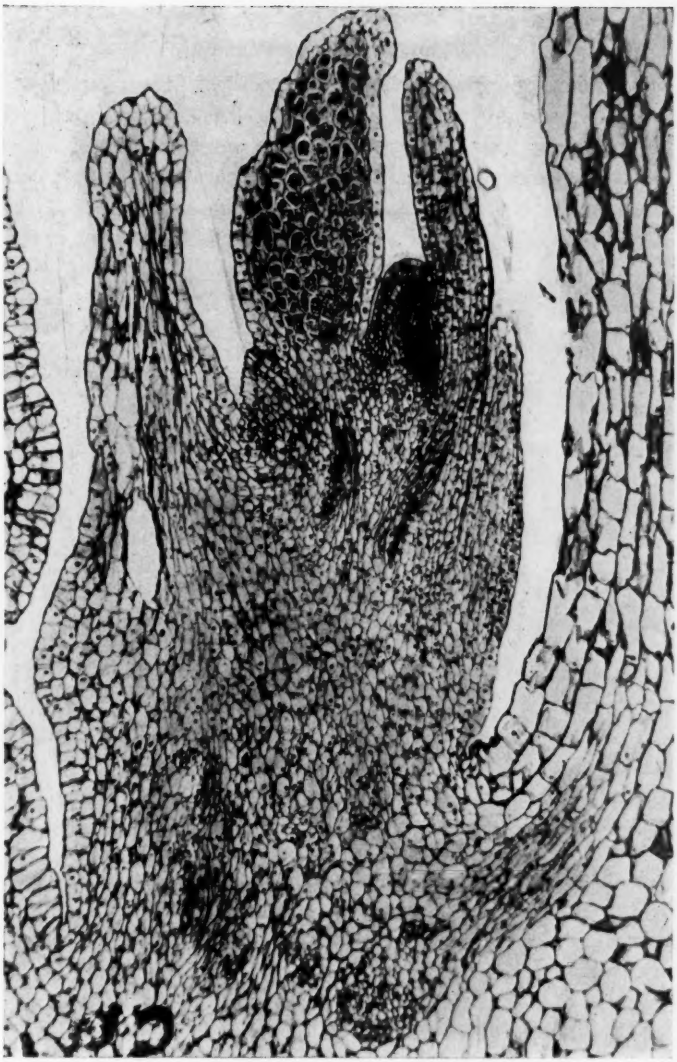

FIG. 15.—*L. harrisii*, still later stage in bud development than in fig. 14, from stem 39 days after treatment. Apical meristem at center surrounded by embryonic leaves.

Cytological observations

The regions of response in the three species of *Lilium* investigated show active and fairly rapid divisions of cells. Mitotic figures from these regions are shown in figure 16. The prophases appear to proceed in an orderly and regular manner. The chromosomes then come to lie in the equatorial plate arrangement characteristic of *Lilium*, and a clearly defined spindle is evident. Each chromosome is composed of two chromatids which separate as the anaphases ensue, and pass through the spindle toward its two poles. The diploid number of chromosomes (24) appears to be present without exception. No evidence indicating the failure of separation of chromatids and no suggestion of fragmentation or other irregularities in behavior have been seen in the cells of either the induced roots or shoots. The observations indicate that the mitotic figures are as distinct as in the cells of root or stem tips taken from plants grown under ordinary garden conditions, and lead to the conclusion that the divisions are perfectly regular in all respects.

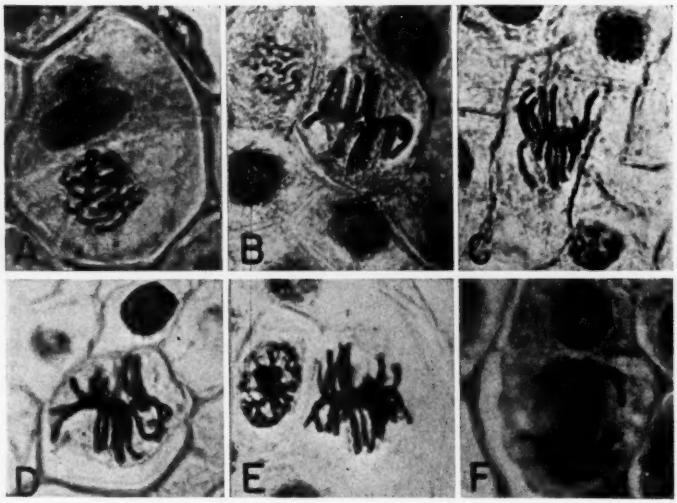

FIG. 16.—Mitotic figures showing regularity of division stages: *A*, *B*, *D*, *F*, from *L. philippinense formosanum; C*, *E*, from *L. harrisii*.

Discussion

Previous reports dealing with the effects of growth promoting substances on higher plants have indicated the inhibition or marked retardation of lateral bud development when the terminal bud is excised and the substance is applied to the cut surface of the stem. In experiments on certain species of *Lilium*, one (*L. harrisii*) has been found in which lateral bud formation may be induced by indoleacetic acid. At the time of treatment the stems showed neither buds nor bud primordia in the leaf axils near the place of application. One to as many as three buds per leaf axil may be induced by the treatment. They develop in the first, second, and/or third leaf axil nearest the surface of application. In the other two species used, *L. philippinense formosanum* and *L. longiflorum*, buds were not induced, but in them adventitious roots were produced from approximately the surface of application to as far down as 2.5 cm. from it. Why such variation in response should occur, and why such marked specificity in the regions affected in the different species should occur, is not understood. The stem anatomy of the three species shows no recognizable structural differences and it is difficult to visualize a different path of transport of the growth substance in the species investigated.

GREENLEAF (4) has recently reported the induction of buds, following similar treatment, in several species and species hybrids of *Nicotiana*. A relatively high percentage of the induced shoots in *Nicotiana* show polyploidy, which is in striking contrast to those produced in *L. harrisii* in which no indications of chromosome doubling or other mitotic irregularities have been detected.

It is possible that the polyploid shoots may arise in *Nicotiana* in the same way that they do in its close relative, the tomato, even if the method which causes callus formation, followed by bud development, in the latter does not induce it in the former. The indoleacetic acid applied to the cut surface of decapitated stems may serve as the excitant for callus formation, and once this is developed, the same conditions or factors which operate to cause chromosome doubling or quadrupling in the tomato may become operative in *Nicotiana*. There is no evidence from the experiments on the bean (10), *Iresine*

(7), tomato (2), or *Lilium* that the indoleacetic acid induces polyploidy.

The histological responses of the cells of *Lilium* to indoleacetic acid are strikingly different in some respects from those in the bean and other plants, which develop large overgrowths or tumors in consequence of the treatment. There is a marked limitation of tissues affected, the cells which respond being limited chiefly either to the fundamental parenchyma in the vicinity of the vascular bundles or to the cells of the outer cortex near the leaf axils. In fact there is a definite specificity of region involved in the two types of responses in *Lilium*. New meristematic regions are generated in response to application of the indoleacetic acid, which develop in two of the species into roots and in the other species into stems.

Cytological observations have been made on the cells of the roots and buds induced by the indoleacetic acid treatment. These have been studied in all possible stages of division in almost all the phases of development of the induced structures. Nothing has been observed which offers support to JONES'S (8, 9) suggestion of the "possibility that missing genes due to corresponding deficiencies in certain parts of both members of a chromosome pair result in a chromosomal unbalance and this brings about unregulated growth," nor was anything seen which suggests a condition of deficiency in any of the chromosomes. Neither were there indications of fragmentation, translocations, or other chromosomal irregularities such as are produced by x-rays, radium, hormones, and other powerful physical and chemical agents. On the contrary there was evidence indicating regularity of division and of chromosome structure and behavior throughout all the stages of mitosis.

Summary

1. The application of 3 per cent indoleacetic acid in lanolin to the cut surfaces of decapitated stems of three species of *Lilium* produced two sharply differing types of response. *L. philippinense formosanum* and *L. longiflorum* responded by the production of adventitious roots from approximately the surface of application to as far down as 2.5 cm. from it, while *L. harrisii* produced buds in the axils of the upper two or three leaves, with no adventitious roots in most cases.

Decapitated untreated stems produced neither adventitious roots nor buds.

2. A few plants of *L. philippinense formosanum* were allowed to develop flower buds. The pedicels of some of the nearly mature buds were cut off about 1 cm. below the receptacles and the cut surfaces smeared with the lanolin mixture. These responded in essentially the same time interval and in much the same way as did the treated stems of this species.

3. A few of the floral buds were permitted to flower. The lanolin mixture was smeared over the unpollinated stigmas of some of the flowers, while in others the ovary was cut across at about 0.5 mm. below the base of the style and the cut surface was then covered with the mixture. Although the treated ovaries enlarged slightly and remained alive for a longer time than did those which were untreated and unpollinated, none of them continued to develop parthenocarpically.

4. Gross observations extending over a period of 3 months were made on treated and untreated stems. Material for histological studies was collected and fixed at 24 hour intervals up to a total of 39 days.

5. In general the three species responded at essentially the same rate, but much more slowly than has been reported for the bean, tomato, and *Iresine*.

6. A small amount of callus may sometimes develop at or on the surface of application, but more often it is wanting.

7. In *L. philippinense formosanum* and *L. longiflorum* the indoleacetic acid produces little or no observable effect upon the cells of the epidermis and outer cortex. The first detectable changes occur in the cells of the fundamental parenchyma, lateral and centrifugal to the vascular bundles. These cells become meristematic, and by repeated divisions give rise to cells which later differentiate as adventitious roots. As a rule only the outer bundles of the stems are involved.

8. As the cells in these regions continue proliferating, and the meristematic masses increase in volume, they encroach laterally and centrifugally upon the fundamental parenchyma which has not responded visibly to the treatment, crushing and digesting these

cells and those of the outer cortex as growth toward the surface proceeds. Before emerging from the stem, a central core of cells is differentiated and an apical histogen becomes evident in the young roots.

9. Following the general blocking out of the root, xylem elements are differentiated near the base and periphery of the inner core of cells. The first xylem differentiated appears always to be tracheids which connect the vascular systems of root and stem. Phloem, which also forms a continuous system between root and stem, is differentiated simultaneously with the xylem but on different radii. New tissues are added from the derivatives of the apical histogen; the root pushes out through the outer cortex and epidermis, and appears normal in all respects.

10. The treated pedicels, while in general responding similarly to the stems, show one rather marked difference. All, or nearly all, of the vascular bundles are associated with the production of adventitious roots. The roots develop in the same way as in treated stems.

11. In *L. harrisii* the region of visible response to the treatment is limited largely to the cells of the epidermis and outer cortex in the immediate vicinity of the leaf axil. The epidermal cells of the stem immediately above the axil elongate radially, while the outer cortical cells centripetal to, as well as slightly below, them enlarge and in the course of about 5 days begin dividing. This marks the initiation of the region at which buds will later develop. Neither buds nor bud primordia are present in the axils of the upper leaves at the time of treatment.

12. The walls of the original parental cells in the regions of response remain distinct, in comparison with those of their derivatives, for a period as long as 12 days. Cell plates, followed by cell wall formation, seem to follow nuclear division in all cases. Multinucleate cells have not been observed in any of the species studied.

13. The groups of meristematic cells in the leaf axils undergo repeated divisions, accompanied by radial divisions of the cells of the epidermis over them. The subsequent growth of their derivatives results in a hump of cells, which through further development and differentiation becomes a bud. From one to as many as three buds may develop in one axil.

14. Vascular differentiation occurs in the induced buds in essentially the same way as in the buds normally produced near the bases of the stems in this species.

15. When permitted to grow on the plants for a period of 8 to 10 weeks, the induced buds develop into bulbils, from the bases of which adventitious roots arise. The bulbils can be removed from the parent plant and grown separately.

16. Cytological study of mitotic figures in both induced roots and buds indicated regular chromosome form and behavior in divisions. There was no evidence of the failure of separation of chromatids, no fragments were observed, and no other irregularities in behavior were detected.

UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

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HISTOLOGICAL RESPONSES OF MIRABILIS JALAPA TO INDOLEACETIC ACID[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 492

KARL C. HAMNER

(WITH TWENTY-EIGHT FIGURES)

Introduction

This paper is one of a series dealing with the gross and histological responses of plants to growth substances, or hormones. A number of dicotyledonous and monocotyledonous plants have been selected, the basis of such choice being the extent to which the particular species or variety responds to such treatment, and the desire to secure a wide range of anatomical types or tissue patterns. When evidence and data gathered in this way are more nearly complete, a more critical and comprehensive evaluation of the phenomena of cellular and tissue differentiation can be made, and many metabolic processes and changes can be studied in greater precision and detail (3, 12, 13, 15).

Previous reports have included studies on the comparative responses of the plant to various growth promoting substances (4, 5, 6, 9, 10, 11), to treatment of various aerial organs of the plant, to treatment of the cut surface of the severed stem as compared with treatment of the uninjured stem, and to treatment of stems of various ages. It is clear (1, 2, 6, 7, 8) that no single tissue or tissue system responds either qualitatively or quantitatively the same throughout the range of types investigated. It is also obvious that in any given species or variety some tissues are much more sensitive to stimulation and respond to a much greater degree than do others. The relative state of maturity of a cell or cells making up a tissue has much to do with the character and degree of its response, as have also specific environmental conditions. Despite these ranges of behavior, however, there is a definite pattern for each species or variety.

[1] This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation. Additional cost of publication sustained by the writer.

Investigation

Most of the material used in this investigation was grown during the late summer and early autumn of 1937. Seeds of four o'clock, *Mirabilis jalapa* Linn., were planted in rich garden soil and grown continuously on an evenly lighted bench in the greenhouse. No attempt was made precisely to control temperature or humidity, although the plants were kept well watered and the walks and benches sprayed with water to prevent excessive temperature or dryness. The plants grew vigorously throughout the time the experiments were conducted.

FIG. 1.—*A:* young plant of *Mirabilis jalapa* at time of decapitation; second internode just starting to elongate. *B:* similar to *A*, 8 days after decapitation and treatment with 2% indoleacetic acid in lanolin.

The specific treatments were made when the plants were of two different ages. One group was treated when the first internode of the young plants had elongated considerably and the second internode had just started to elongate (figs. 1*A*, 3). Another group was treated when the plants were older, the fourth internode having elongated and the fifth just beginning to elongate (figs. 2, 23). In all instances here recorded, treatment consisted of completely severing the stem in the upper portion of the first internode. Stems of the first type showed no outer peripheral secondary thickening, those of the second an appreciable amount (figs. 3, 23). Immediately after such decapitation the cut surface of most of the plants was treated with a small amount of a 2 per cent indoleacetic acid-lanolin paste. A few of the plants were treated with pure lanolin only, and

some were left with the cut surfaces uncovered and exposed. Still other plants were treated with a small ring of the lanolin mixture completely encircling the uninjured stem, which was not decapitated. Since these plants showed the same general types of histological responses, with the exception that the pith parenchyma was much less active than in those decapitated and treated apically, the details are not recorded here. The effects, in so far as they differed from the decapitated plants, were of the same general kind as already indicated for the bean (**6, 8**) and *Iresine* (**7**), in which the same types of technique were employed.

Material for histological investigation was collected from each group of plants at various intervals after decapitation and treatment. Navashin's solution was used throughout as a fixative. The butyl-alcohol paraffin method of imbedding was used, and the material sectioned at 10 μ.

Gross responses to treatment

The gross responses of the treated stems were similar, whether the fourth internode or only the first was elongated at the time of treatment. Within twenty-four hours the tissues near the treated surface showed a distinct yellowing; at forty-eight hours the tip for a distance of 1 to 1.5 mm. from the treated surface had increased in diameter more than the remainder of the stem. By the end of six days enlargement had increased appreciably and root primordia were visible in vertical rows beneath the surface. At nine days still greater swelling had taken place and the tumor was light yellow or almost white, its surfaces more or less tuberculated, and the young roots were protruding not only from its lateral surfaces but often also from the upper surface where the lanolin mixture had been applied. After the fifteenth day the tumor may continue to enlarge slightly and the young roots protrude somewhat farther, the degree of such emergence and continued development depending directly upon the atmospheric humidity. If the air is nearly saturated, the roots become long and threadlike; if it is dry they protrude scarcely beyond the surfaces of the tumor and many of them do not emerge at all. The tumor may continue growth for at least one hundred days, but during this time there is no great proliferation of tissues

FIG. 2.—Young plant with fourth internode elongated. Such plants were severed near top of first internode and treated with lanolin mixture. They differ mainly from those shown in fig. 1 in that secondary thickening in treated region is somewhat advanced and all primary tissues are more nearly mature (see figs. 23, 24).

to produce a large apical tumor above the treated surface, as in the bean. Although the lateral shoots in the axils of the cotyledons are inhibited in development for several days, they generally grow rapidly after the sixth day following application of the lanolin mixture. After growth has been initiated they develop much as do shoots in untreated plants, and overlap the decapitated central axis with its terminal tumor which, as stated, may persist for many weeks but does not usually become more than 1 cm. in diameter. In some plants, however, the lateral shoots may be almost completely suppressed. In such instances the cotyledons become thick and fleshy, and persist for at least four or five months. The hypocotyl increases markedly in diameter, much more rapidly than in decapitated untreated plants, and also more than in check plants which were not decapitated but allowed to grow without any type of treatment other than watering. The tumors on these plants generally become relatively large, more or less globular, green, with scurfy surfaces. Two of them attained a diameter of more than 2 cm. by the end of five months, and were apparently still growing when harvested. None of the plants was given more than the single initial treatment with the lanolin mixture. The tumors seldom extend for any considerable distances down the stem. They are more or less flat topped, sides bulging slightly, and these terminate below rather abruptly. Below this sharp taper the stem as a whole shows very little change from the untreated stem of a non-decapitated plant, except that when lateral shoots develop from the cotyledonary node, the first internode (except for the tumor) does not continue to undergo secondary thickening so rapidly nor to so great an extent as that of the undecapitated plant.

The first internodes of plants decapitated when the second internode is just beginning to elongate and treated with pure lanolin never produced tumors. None of them increased in diameter following decapitation nor showed any additional secondary thickening. Some of them developed a very weak phellogen-like layer just below the treated surface, remained green for about two weeks, and then the whole first internode became excised following formation of an abscission layer just above the cotyledonary node. All of the decapitated and untreated first internodes became abscised in this way

within two or at most three weeks following decapitation. There was practically no subsequent increase in cell number or cell size, although the internodes remained green and turgid for seven to ten days. After that time they became yellowed and dropped from the plants either while still fairly turgid or after becoming very much shriveled (fig. 6*A*, *B*). In every instance shoots developed in the axils of the cotyledons and after three or four weeks the cotyledons became yellow and gradually withered or were abscised.

Histological details

Anatomy of untreated stems

Mirabilis jalapa is similar in stem anatomy and in type of secondary thickening to forms in the Chenopodiaceae and Amaranthaceae which have been described by Wilson (**16**). At the time the first internode has elongated considerably and the second internode has elongated but slightly (fig. 1*A*), a cross section of the first internode just below the second node shows very little secondary thickening (fig. 3). The stele is bounded peripherally by an endodermis or starch sheath. The pericycle consists of a layer or two of cells just inside the endodermis. Roughly there appear to be two sets of vascular bundles, each set arranged in the form of an ellipse. The inner ring consists of from eight to ten larger vascular bundles surrounded by or scattered through the pith. The outer ring consists of many smaller bundles some of which lie just inside the pericycle and others several cell layers inside the pericycle. Each of these bundles may possess a fascicular cambium from which both secondary xylem and phloem may be derived, although usually the amount is not great. Between each of these smaller bundles are a number of parenchymatous cells, and from these cells an interfascicular cambium may be developed, a cambium becoming continuous laterally either with the fascicular cambium of some of the smaller vascular bundles, with a cambium derived from the pericycle over the phloem of such a bundle, or with both such cambiums (fig. 4).

There is very little cambial activity in the vascular bundles in which the fascicular cambium is not continuous with the interfascicular cambium, but that portion of the cambium which is

present in the small outer bundles and which is continuous with the interfascicular cambium may be much more active and considerable secondary xylem and secondary phloem may be differentiated from it (fig. 5). The interfascicular cambium itself is highly meristematic, and from its derivatives there differentiate the conjunctive tissue masses of isolated tracheae and tracheids, isolated phloem elements, and additional vascular bundles with an active cambium remaining between the xylem and phloem portions (fig. 5). At about the time the fourth internode has elongated, a cross section of the first internode shows very little secondary thickening in any of the bundles except for the outermost ones (figs. 23, 24), which have an appreciable amount of secondary xylem and phloem, and between them there is a considerable amount of conjunctive tissue.

Shortly after the stage shown in figures 23 and 24, the activity of the fascicular cambium may cease in some of the vascular bundles and a cambium develops in the pericycle over the vascular bundles and becomes continuous with the interfascicular cambium (fig. 4). Division continues in the entire ring of cambium around the stem (figs. 4, 5). Under ordinary circumstances the cambium in the larger bundles of the ellipse nearer the center of the stem is never highly meristematic; even stems six to eight months of age, as shown in figure 5, have very little of either secondary xylem or phloem. In stems treated with lanolin mixture there is much less response by the cambium in these bundles than in the peripheral cambiums.

If the stem is severed across the first internode at the time the second internode is just beginning to elongate and the cut surface is not treated or is treated with lanolin only, there is practically no additional meristematic activity in the remaining cells of the first internode (fig. 6). They may remain alive and the internode change little from the time of decapitation (fig. 6*A*) until it is abscised, or many of its cells may die and the whole stem become shriveled (fig. 6*B*).

CHANGES IN PLANTS TREATED WHEN ONLY FIRST INTERNODE HAS ELONGATED

Following decapitation and treatment of the cut surface with the lanolin paste, little histological response was shown during the first

two and one-half days. The portion of the first internode below the treated surface appears in cross section (fig. 7*A*) very similar to the plant at the time of treatment (fig. 3). Some of the cells of the pericycle and interfascicular parenchyma show rather large nuclei and dense cytoplasm. Four days after treatment (figs. 7*B*, 8*A*, *B*) marked changes have taken place a short distance below the treated surface. Cells of the cortex, pericycle, and interfascicular parenchyma in some portions of the stem are actively dividing (figs. 7*A*, 8*B*); in other portions little change is apparent (fig. 8*A*). Some divisions have taken place in cells of the endodermis, and in cells of the pith nearer the center of the stem.

Subsequent developments are indicated in figures 7 to 21. Studies of a number of plants were made at regular intervals following treatments. Only those examples which show significant progressive changes are shown. In general there was decided uniformity in response but not every plant responded at the same rate. Certain of the plants, although only the first internode had elongated at the time of treatment, had much thicker stems than had others, and these larger stemmed plants responded more rapidly than those with a stem of smaller diameter. A careful study of the type of response, however, showed no essential difference in the pattern. Both types of plants have been used for the illustrative material.

In every case the portion of the stem from 1 to 3 mm. below the treated surface responded most rapidly, resulting in a spindle-shaped tumor in about seven days. From this region most of the adventitious roots appeared. Subsequently tissues of the pith adjacent to the treated surface began active division, producing masses and small tubercles of tissue extending above the plane of the original cut surface. By fourteen days following treatment the tumor was roughly hemispherical. In practically every case, the only response to the treatment exhibited by the portion of the stem more than 6 mm. below the treated surface was growth and development of a type such as might occur if the plant had not been decapitated and treated. Except for the consideration of the responses of the pith, the discussion of the responses of the separate tissues which follows will be confined to the response in that portion of the internode from 1 to 3 mm. below the treated surface. If a response was

obtained above or below this region in a given tissue, it was usually of lesser degree.

In none of these experiments has the epidermis shown marked response to the treatment. The cells may enlarge somewhat and few radial divisions may occur, but no tangential divisions have been observed. As the result of the growth of tissues underneath it, the epidermis may be stretched and ruptured and a few weeks after treatment portions of it may be dead.

The cortex shows definite response but, except for the endodermis, never becomes highly meristematic. Many divisions may occur throughout the cortex, but even in the largest tumors it does not constitute a conspicuous portion of them. As the result of active cell division and growth in the endodermis and stele, portions of the cortex may be ruptured and many of its cells die. In no case was there observed a cork cambium such as appears in the cortex of old, untreated, and undecapitated plants (fig. 5).

The cells of the endodermis divide and produce many parenchymatous cells as a result of treatment. By far the greatest number of cell divisions occur in that portion of the endodermis peripheral to a developing adventitious root, and these are largely radial, the endodermis forming a layer of meristematic tissue capping the tips of the young roots (figs. 9, 10, 11, 13). Activity in the endodermis never becomes so great as that in the tissue just inside it, nor does it form a wide band of meristematic tissue. Derivatives from it have not been observed to differentiate as strands of xylem and phloem as in red kidney bean (**8**).

Perhaps the most sensitive tissues are the pericycle and the interfascicular parenchyma. Divisions in the cells of the pericycle were apparent within four days after treatment (figs. 7*B*, 8). Many of the adventitious roots formed in response to the treatment were differentiated almost entirely from the derivatives of this tissue (figs. 8, 11). Other adventitious roots were differentiated in a mass of meristematic tissue containing derivatives of both the pericycle and interfascicular parenchyma. This reaction of the pericycle is in contrast to its limited response in red kidney bean (**6, 8**) and tomato (**3**).

The interfascicular parenchyma lying just within the pericycle

and between the outer ring of vascular bundles is about equally as sensitive as the pericycle. It is in this tissue that the interfascicular cambium of undecapitated and untreated plants develops. Its cells have become actively meristematic within four days after treatment, and form, together with the meristematic band produced by the pericycle, a mass of meristematic tissue in which many adventitious roots are differentiated and which grow outward through the cortex and epidermis. Other derivatives of the proliferation of the interfascicular parenchyma may remain parenchymatous, or they may form provascular strands which differentiate as vascular bundles connecting the stele of the developing roots to the vascular tissues of the stem, or they may form vascular strands connecting those present in the stem at the time of treatment.

The fascicular cambiums of the various bundles of the stem are not equally responsive. There is little if any response noticeable on the part of the cambium of any of them except that present in the small outer bundles which abut the pericycle. In these the cambium, which corresponds to that portion of the fascicular cambium of undecapitated plants which becomes continuous with the interfascicular cambium, is more sensitive. Following treatment it proliferates, producing a broad band of parenchymatous cells between the xylem and phloem. The xylem and phloem of these small bundles may become crushed as the result of growth and division in the cells around them (fig. 11). In other instances the fascicular cambium shows very little activity (figs. 13, 16), and occasionally additional phloem and xylem elements are differentiated, although frequently no more than in untreated stems.

The cells of the xylem and phloem of the bundles respond little or not at all to the treatment. Some of the parenchymatous cells may enlarge somewhat, but such divisions as occur are very few. This behavior, too, is in marked contrast to the bean and tomato.

Cells of the pith respond more slowly than do cells of the pericycle, interfascicular parenchyma, and the cambium of the small outer bundles; but after activity has been initiated the cells proliferate rapidly and activity continues over a period of many weeks (figs. 12, 18, 21). A few divisions may be observed the fourth day after treatment but the greatest activity begins about the seventh

day (fig. 13). At this time many root primordia have been differentiated in the peripheral portions of the stele. Shortly after root primordia are discernible in derivatives of the pericycle and interfascicular parenchyma, provascular strands may be formed from the derivatives of the pith and these strands connect the stele of the developing root with both the inner and outer vascular bundles of the stem (figs. 13, 20). At about this time divisions of cells throughout the pith may take place, but the greatest activity occurs in those cells surrounding the vascular bundles and in the provascular strands which have differentiated between and interconnecting various vascular bundles of the stem. In this way a complex anastomosis of provascular strands, stem bundles, and adventitious roots is established (figs. 17, 20). Some of these derived vascular bundles run longitudinally in the stem parallel to the original bundles, and are closely anastomosed with them (fig. 15). In some stems adventitious roots are differentiated from the derivatives of actively dividing pith cells adjacent to the outer vascular bundles (fig. 18) or the inner vascular bundles (figs. 22, 27, 28). Such root primordia may differentiate centripetally, laterally, or centrifugally to the bundle and the derived root may grow in almost any direction through the pith, through the surrounding tissues, or outwardly (fig. 18).

The cells of the pith adjacent to the vascular bundles continue meristematic activity for many weeks, and the cells so derived may appear as lumps and overgrowths of intermingled parenchymatous and vascular tissue on the upper surface of the treated portion (fig. 19). Except for the differentiation of roots from derivatives of the pith, this tissue responds much as did the pith of red kidney bean, and constitutes the main body of the very large old tumors previously mentioned.

Responses of plants treated when fourth internode has elongated

In general, those plants which were decapitated and treated at the time the fourth internode had elongated did not produce so vigorous a response as did the younger plants. At the time of treatment the first internode of these plants (figs. 23, 24) possessed a considerable amount of secondary tissue, whereas the smaller plants had

very little (fig. 3). This secondary tissue consisted almost entirely of an outer band of conjunctive tissue. There was some secondary xylem and secondary phloem in the smaller outer bundles whose cambium was continuous with the interfascicular cambium (fig. 24). All other bundles of the stem had very little secondary tissue.

The most sensitive tissues of these stems were the pericycle, the pericyclic cambium, and the interfascicular cambium. As the result of rapid divisions in these three tissues, within six days after treatment there were masses of meristematic tissues in areas lying between the endodermis and the band of conjunctive tissue. In these masses root primordia were differentiated (fig. 25). In some instances the cambium of one of the smaller outer bundles responded by active cell division, in which case its derivatives took part in the differentiation of developing roots. In all examples in which roots were developed in the tissues just described, differentiation of provascular strands from the stele of the root proceeded inward only as far as the band of conjunctive tissue (fig. 26). No vascular connections were observed between these roots and the bundles centripetal to the conjunctive tissue.

The tissues of the epidermis, cortex, and endodermis responded in much the same manner as described for the younger plants. In contrast to the response of the latter, there was no observable response of the interfascicular parenchyma which might still persist central to the conjunctive tissue. There was no apparent response of the tissues of the vascular bundles with the exception of the cambium of a very few of the small outer ones, as has previously been described.

The pith responded to treatment in a manner similar to that of corresponding areas in the younger plants. The cells surrounding the vascular bundles were the most sensitive and as a result of active divisions of them and in localized areas of other portions of the pith, strands of meristematic tissue extending from bundle to bundle and longitudinally in the stem were formed and these later differentiated as vascular bundles. In certain regions derivatives of the pith cells adjacent to the vascular bundle became organized as root primordia (figs. 27, 28), which upon development formed roots extending in various directions through the pith.

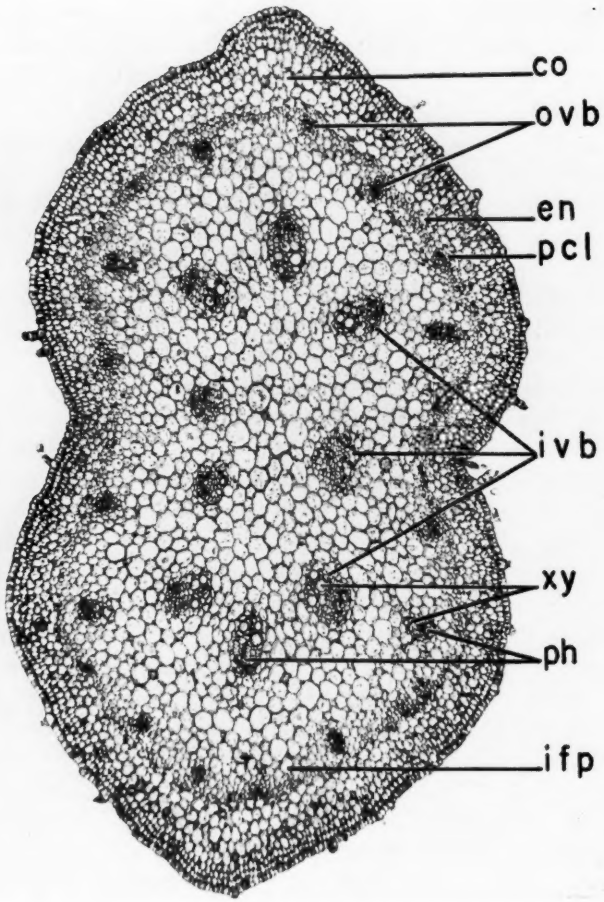

FIG. 3.—Cross section of first internode of plant as shown in fig. 1*A*. *co*, cortex; *en*, endodermis; *pcl*, pericycle; *xy*, xylem; *ph*, phloem; *ivb*, inner vascular bundles; *pi*, pith; *ovb*, outer vascular bundles; *ifp*, interfascicular parenchyma.

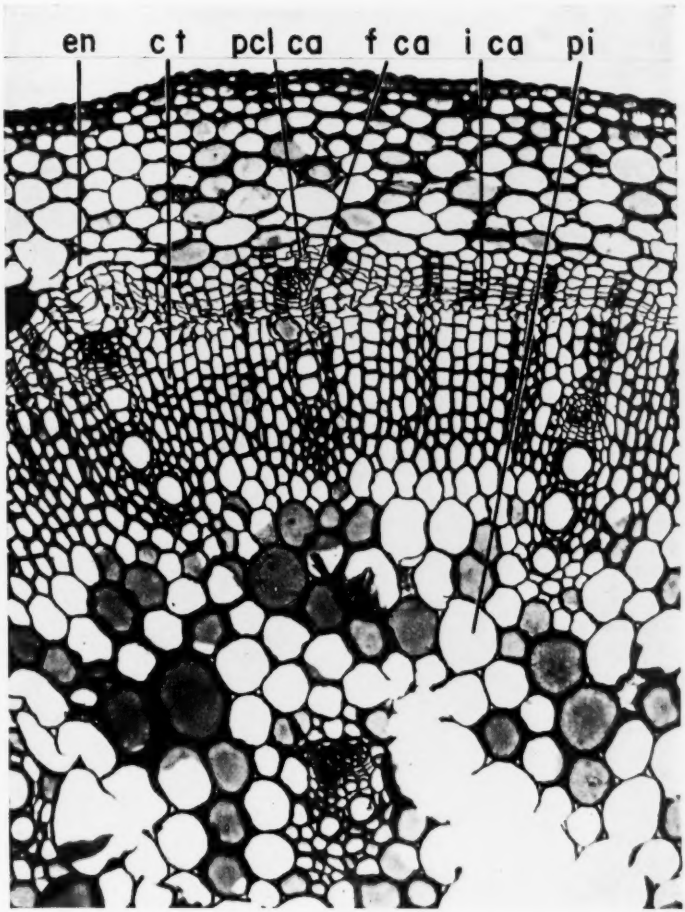

FIG. 4.—Cross section of first internode of untreated plant sixth internode of which is elongating. Considerable conjunctive tissue has been derived from interfascicular and pericyclic cambiums, and secondary xylem and phloem from fascicular cambium of some of outer bundles. A cambium, continuous with the interfascicular cambium, has been derived from the pericycle over the two bundles at upper left hand. *en*, endodermis; *ct*, conjunctive tissue; *pcl ca*, pericyclic cambium; *f ca*, fascicular cambium; *i ca*, interfascicular cambium; *pi*, pith. Inner bundle at lower center showed but few secondary elements derived from cambium.

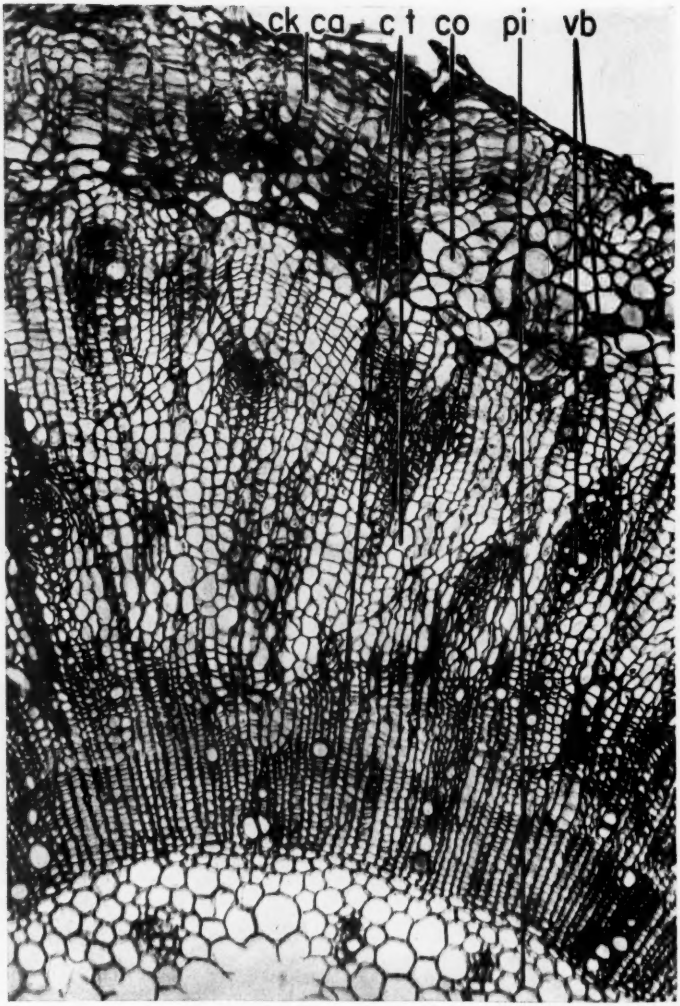

FIG. 5.—Cross section of basal region of stem several months old. *ck ca*, cork cambium; *ct*, conjunctive tissue; *co*, cortex; *pi*, pith; *vb*, vascular bundle. Most of secondary tissue of stele derived from pericyclic and interfascicular cambiums.

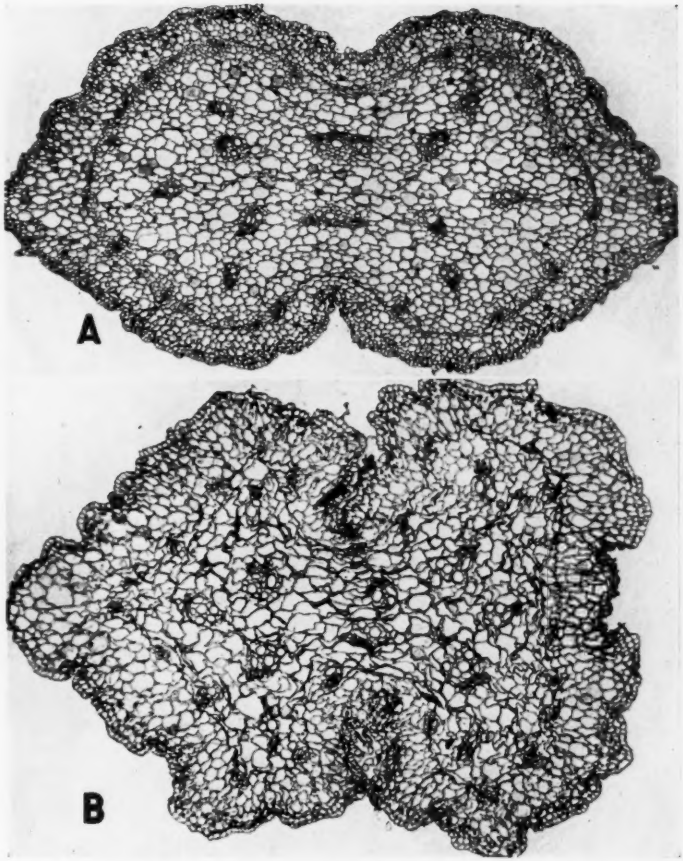

FIG. 6.—Cross sections of untreated first internodes 13 days after decapitation. *A:* most cells of tissues have remained alive but there has been little meristematic activity and no secondary thickening. *B:* same type of stem; many cells dead and collapsed. *B* is slightly more highly magnified than *A*. *Cf.* fig. 18 which is about the same age but had been treated with lanolin mixture, and fig. 23 which is also about the same age but was not decapitated or otherwise treated.

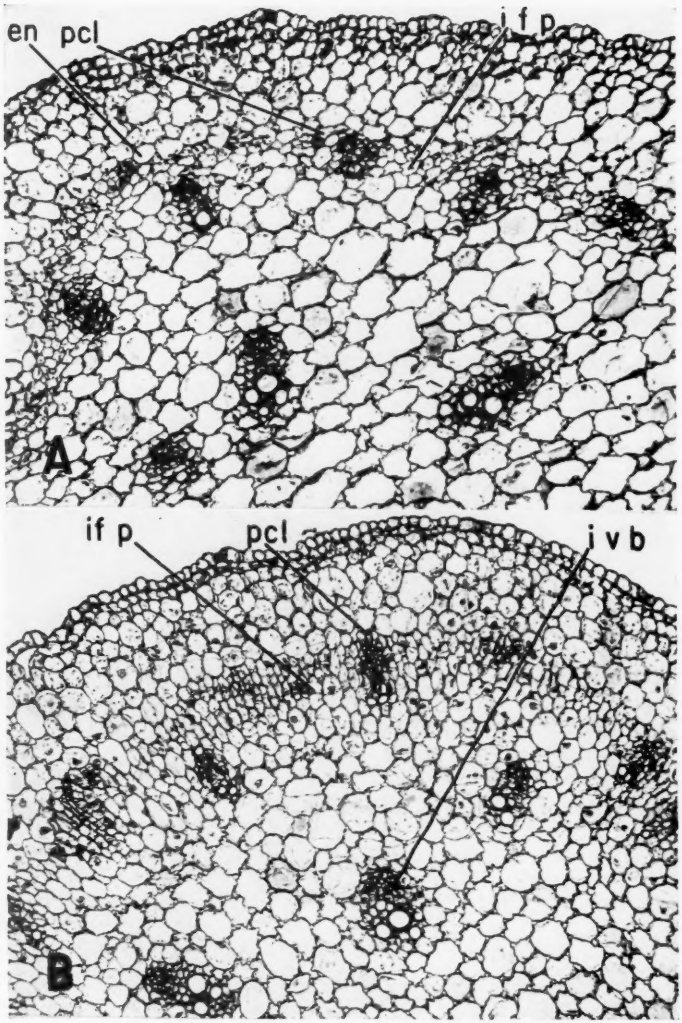

FIG. 7.—*A:* 60 hours after treatment, section about 1 mm. below treated surface; some activity of fascicular cambium and of phloem parenchyma. *B:* 96 hours after treatment, section about 1 mm. below treated surface. Cells of phloem, cambium, pericycle, and interfascicular parenchyma actively dividing. Some divisions in endodermis, cortex, and pith. *en*, endodermis; *pcl*, pericycle; *ifp*, interfascicular parenchyma; *ivb*, inner vascular bundle.

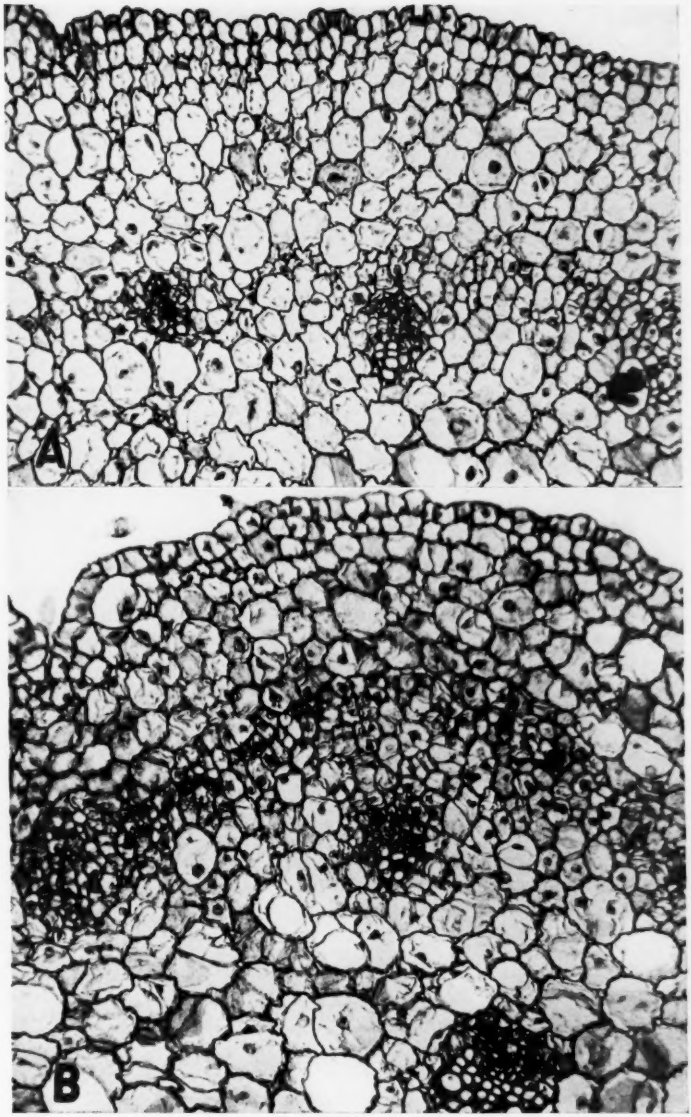

FIG. 8.—Ninety-six hours after treatment, 1 mm. below treated surface. *A:* sector of stem showing relatively little activity. *B:* of same section showing much greater activity; interfascicular parenchyma and pericycle especially active.

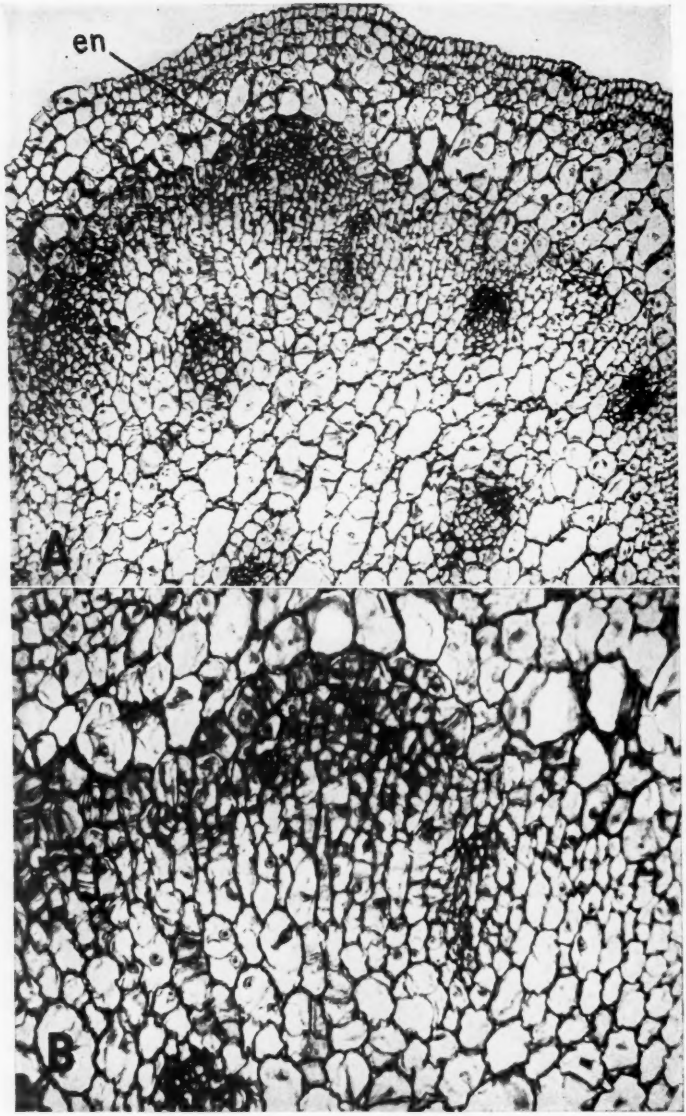

FIG. 9.—Five days after treatment, showing beginning of root primordium as derived mainly from interfascicular parenchyma and pericycle. Endodermis actively dividing; its derivatives form a cap over the primordium. *B:* enlarged view of portion of *A*.

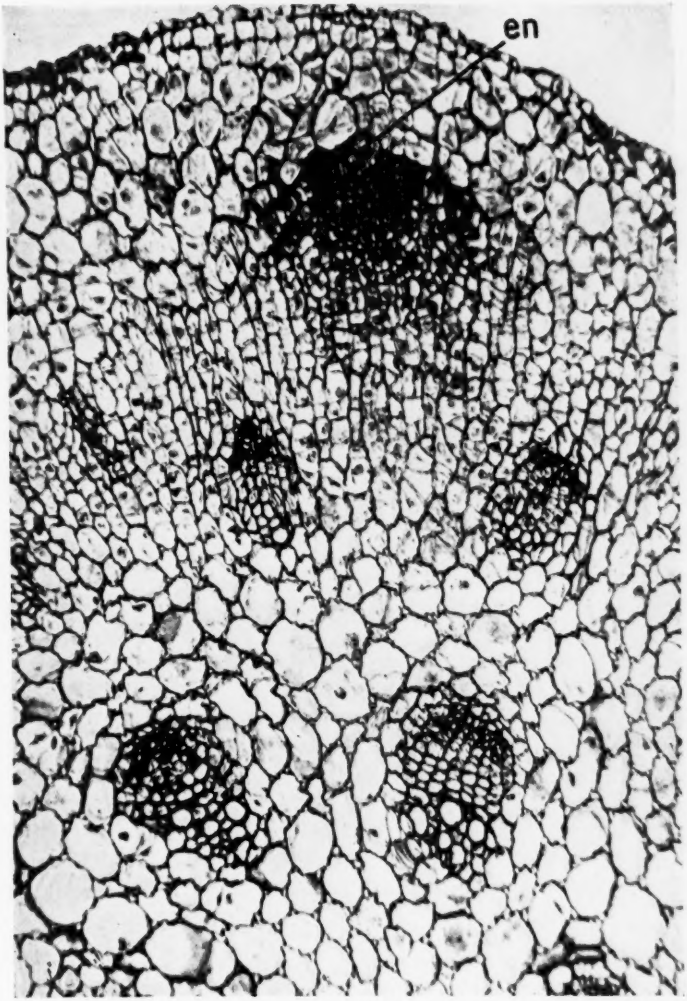

FIG. 10.—Six days after treatment, 1.5 mm. below treated surface. Root primordium from derivatives of pericycle and interfascicular parenchyma. Little activity of pith or cells of vascular bundles. *en*, endodermis.

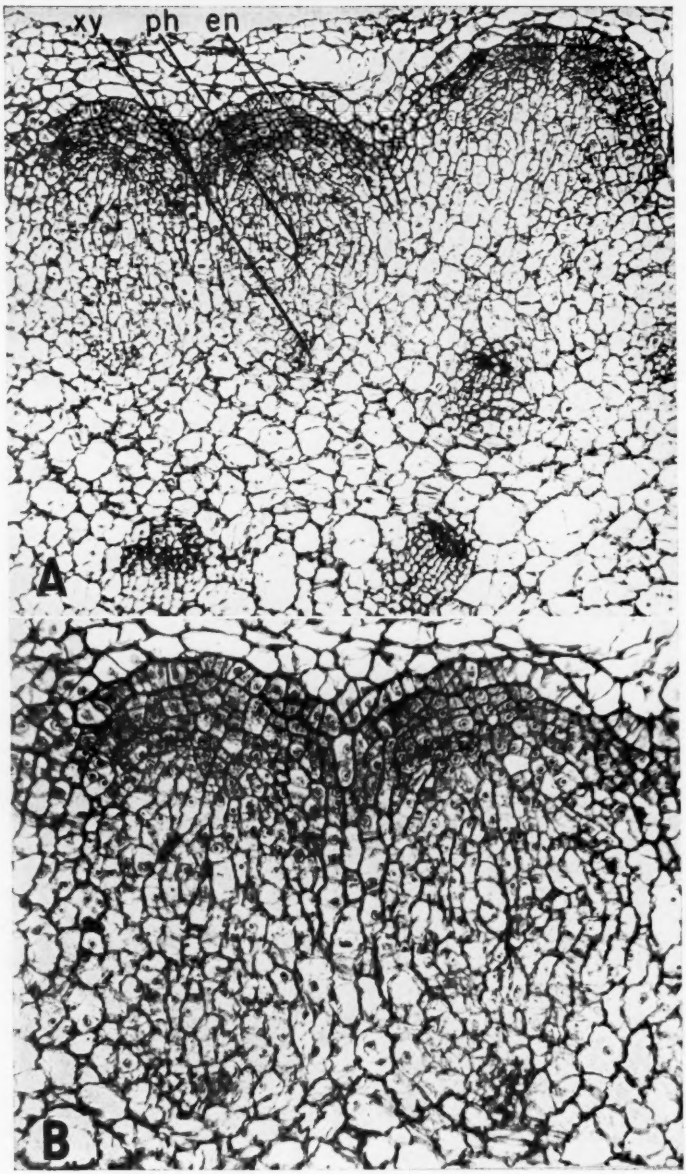

FIG. 11.—Six days after treatment, section about 1.5 mm. below treated surface. *A:* sector showing three root primordia directly over small outer vascular bundles and differentiated mainly from derivatives of pericycle. Phloem and xylem of two outer bundles at left separated by active cells; such activity not manifest in outer bundle at right nor in inner bundles. *en*, endodermis; *xy*, xylem; *ph*, phloem. *B:* section of *A* enlarged.

FIG. 12.—Median longitudinal section, 6 days after treatment. Activity greatest 1.5 to 3 mm. below treated surface. Three root primordia shown.

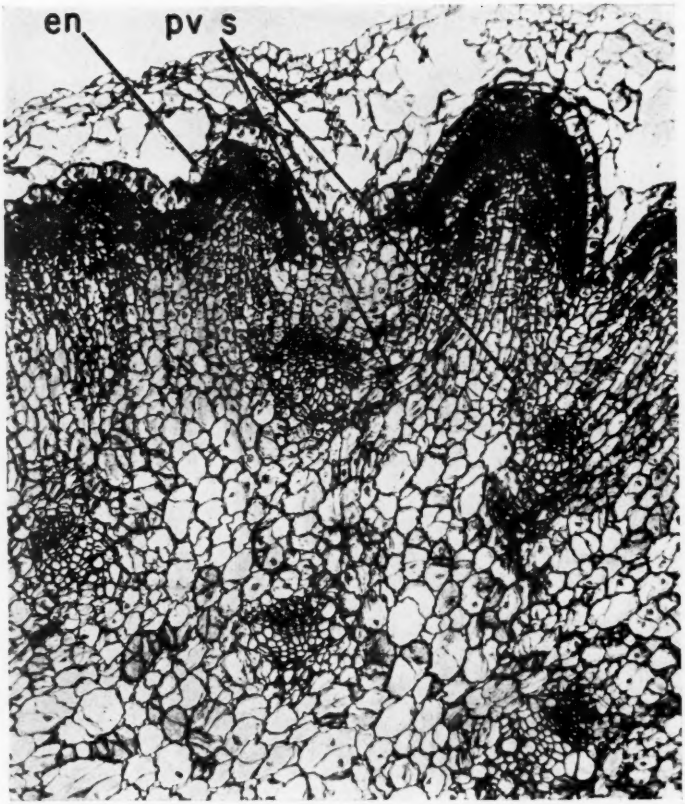

FIG. 13.—Seven days after treatment. In root at upper right, provascular strands differentiating to two outer vascular bundles adjacent to interfascicular parenchyma from which root was mainly derived, and also inward to inner bundle at lower center. Parenchymatous cells around the various vascular bundles actively dividing. *pvs*, provascular strands; *en*, endodermis.

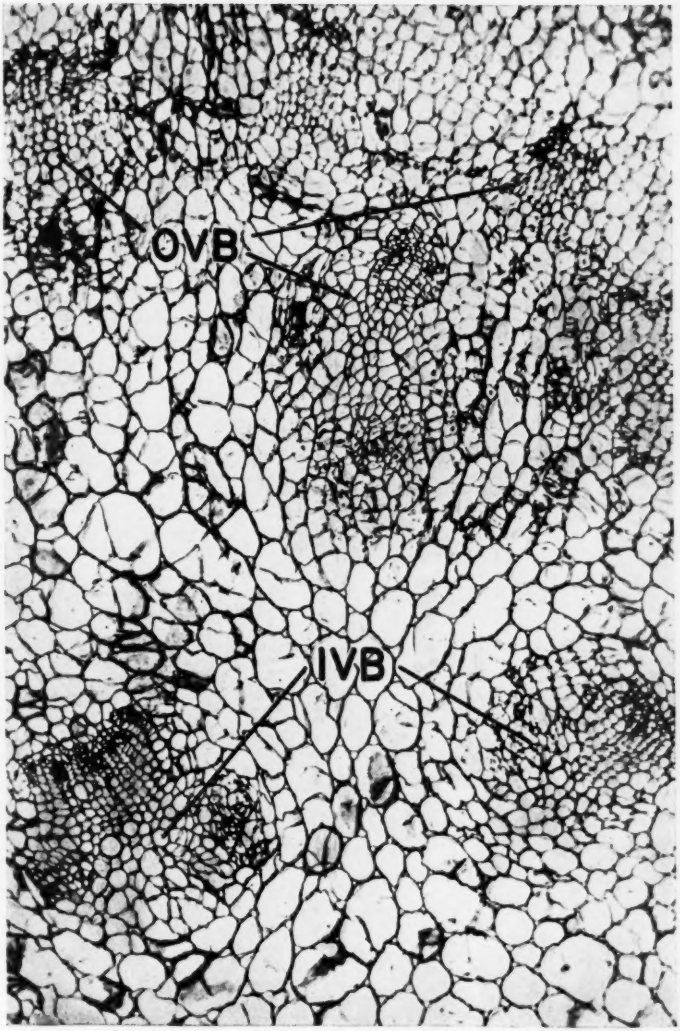

FIG. 14.—Nine days after treatment. Enlarged view of small portion of stem showing two inner vascular bundles and several outer ones. Parenchyma surrounding vascular bundles has actively divided, resulting in strands or islands of meristematic tissue; some derivatives have matured as scattered phloem and xylem elements, and strands adjacent to xylem of original bundles. *ivb*, inner vascular bundles; *ovb*, outer vascular bundles.

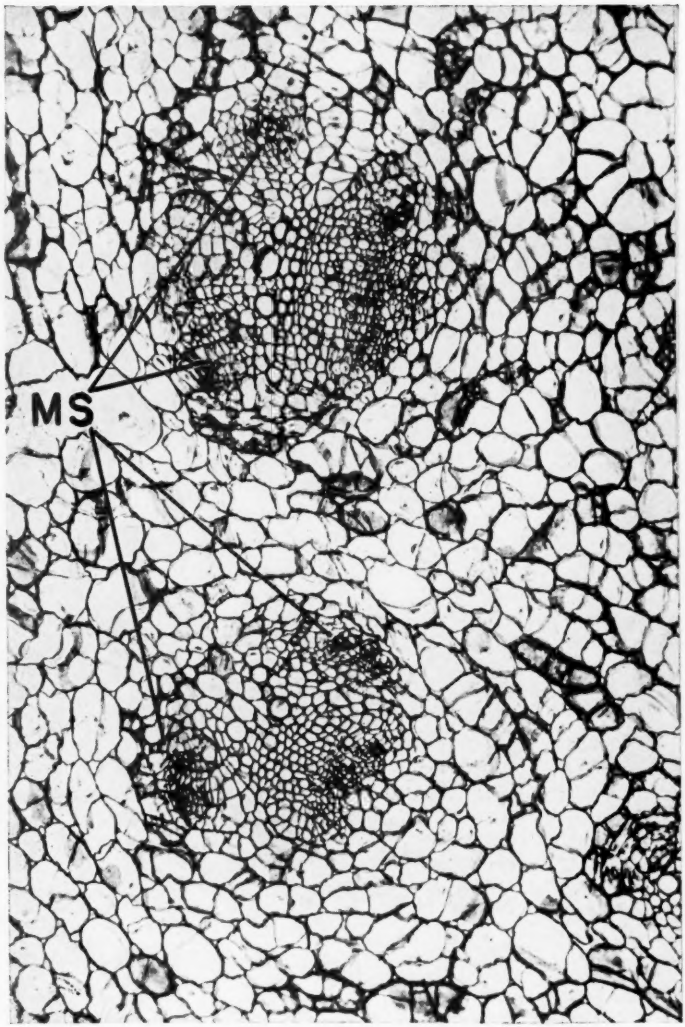

FIG. 15.—Enlarged view of meristematic pith and two of inner vascular bundles similar to those in fig. 14; vascular strands differentiated from derivatives of pith. Cambium of original inner vascular bundles only slightly active.

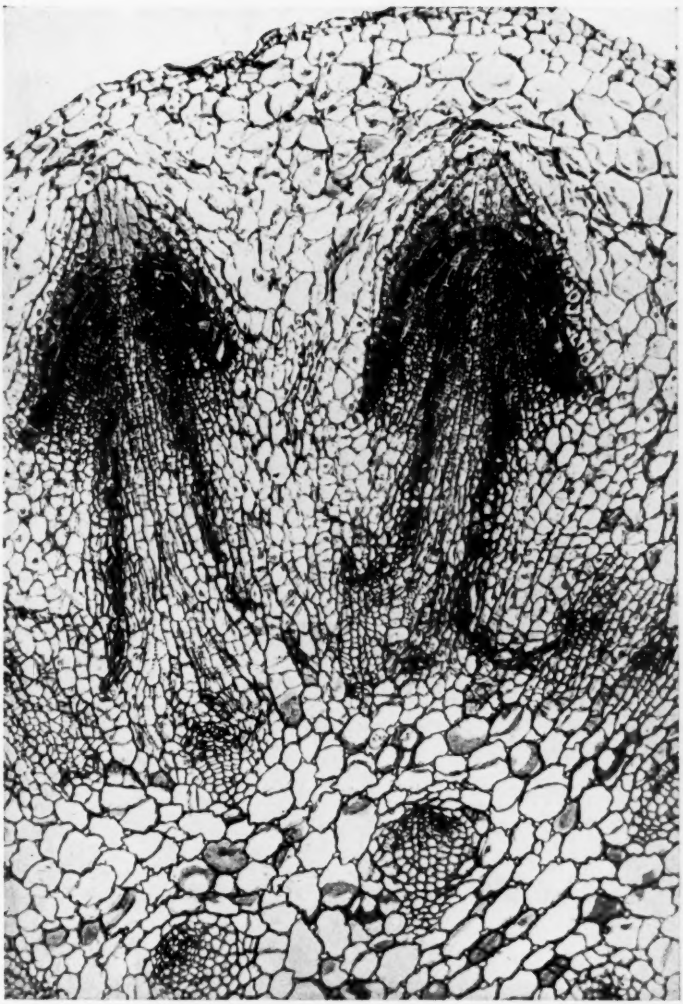

FIG. 16.—Eleven days after treatment. Root on left has developed directly centrifugal to one of small outer vascular bundles and is comparable with primordium shown at right in fig. 11*A*. Differentiation of strands of tracheids around phloem of a bundle to the xylem of the same bundle. Root on right has developed from interfascicular parenchyma between two of the outer vascular bundles and is comparable with primordium shown at right in fig. 13. Vascular connection established to each of the two adjacent bundles.

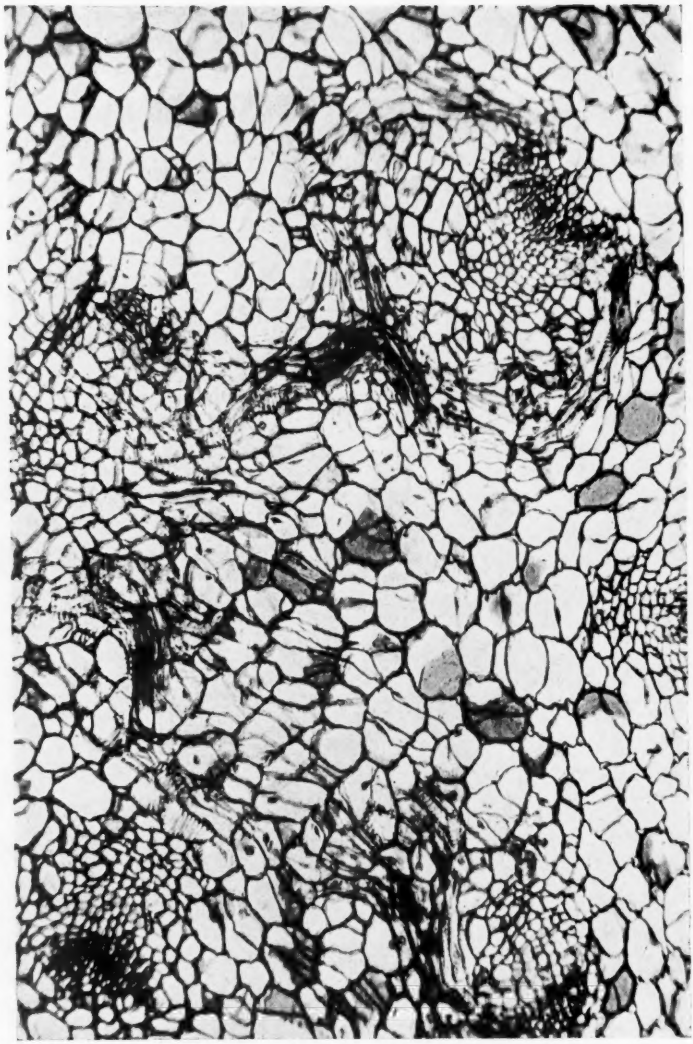

FIG. 17.—Eleven days after treatment. Meristematic strands from which tracheids and other xylem and phloem elements are being differentiated extend in various directions, establishing vascular continuity from bundle to bundle and longitudinally up and down the stem. *Cf.* fig. 15.

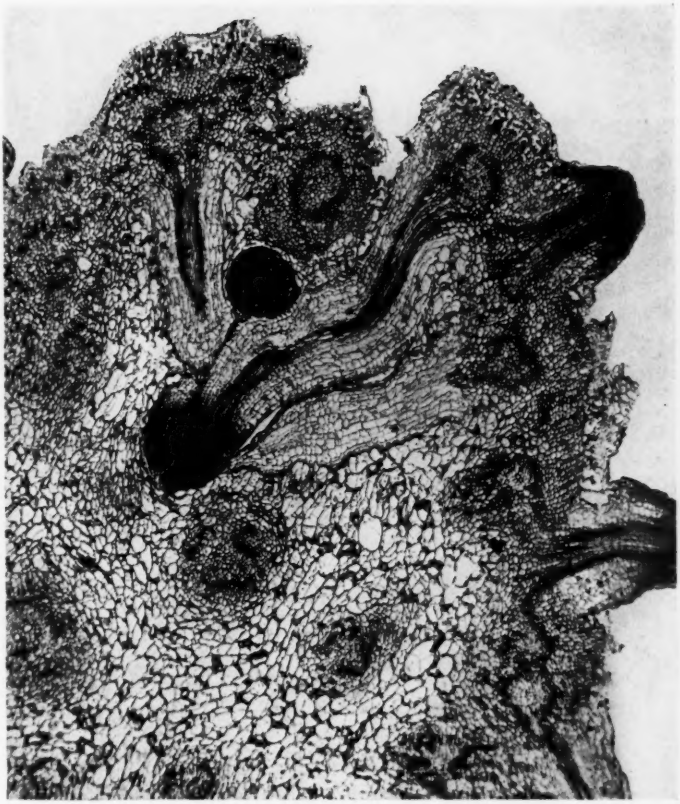

FIG. 18.—Cross section, 13 days after treatment. In addition to roots developing outwardly, others have been initiated from derivatives of parenchyma adjacent to xylem portions of outer vascular bundles, and have penetrated inward toward center of stem.

FIG. 19.—Median longitudinal section of stem 17 days after treatment. In addition to numerous young roots in longitudinal series down the stem, pith surrounding the vascular bundles is highly meristematic.

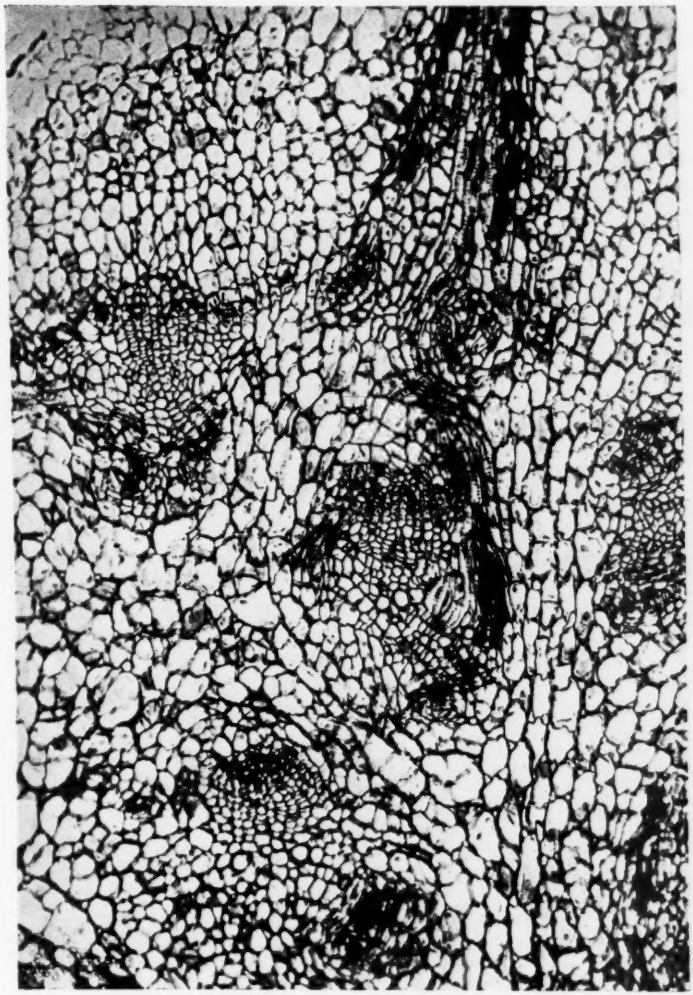

FIG. 20.—Twenty-two days after treatment. Cross section of portion including base of root and several vascular bundles. Vascular connections have been differentiated from the root to the vascular bundle at center and to a small bundle adjacent to it, the adjacent bundle having been differentiated from derivatives of pith near xylem of stem bundle, as shown in figs. 15 and 21.

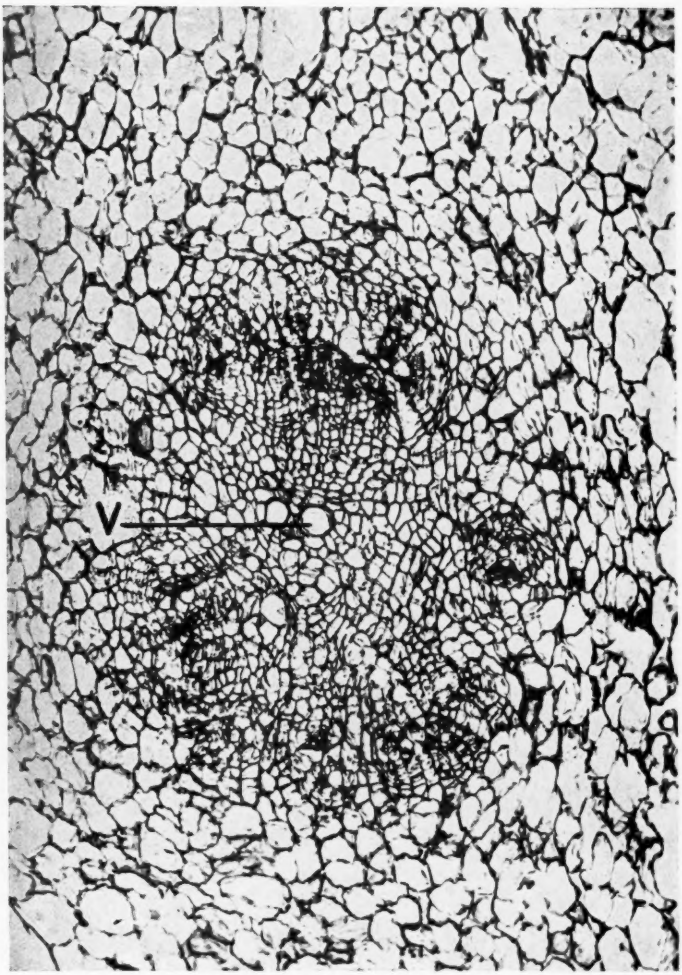

FIG. 21.—Twenty-eight days after treatment. Cross section of portion including one of inner vascular bundles. Parenchymatous cells surrounding bundle are highly active, especially those adjacent to vessels of primary xylem. From derivatives of these cells strands of meristematic cells and vascular elements have been derived. Parenchymatous cells in bundle are slightly active. *v*, vessels.

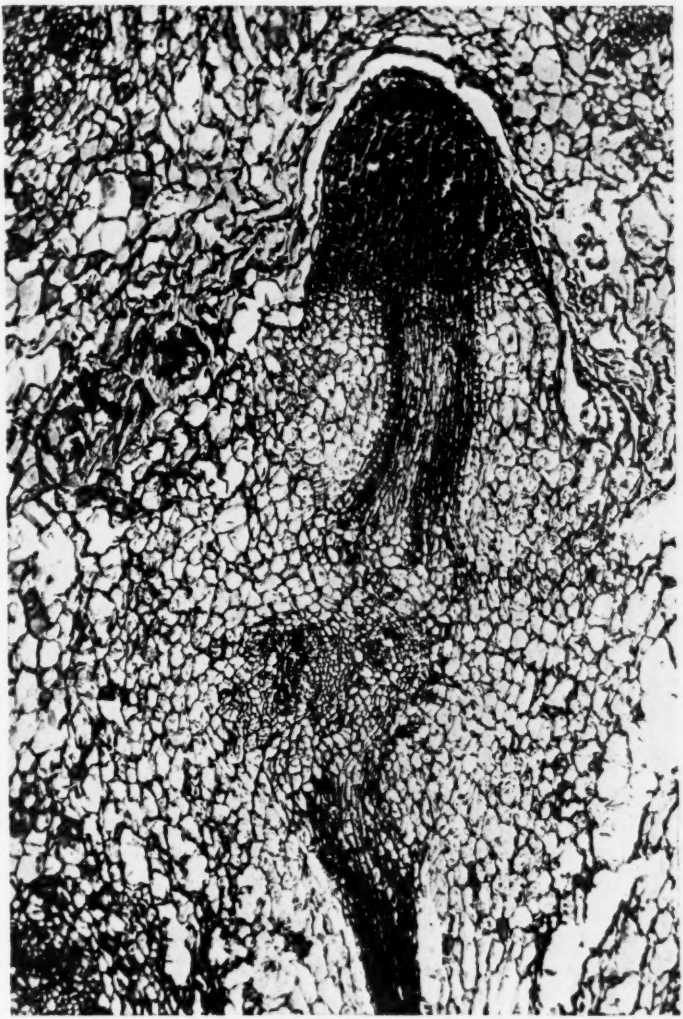

FIG. 22.—Twenty-eight days after treatment. Parts of two roots shown, each of which has developed from derivatives of actively dividing pith parenchyma located on either side of inner vascular bundle shown at center. *Cf.* fig. 18.

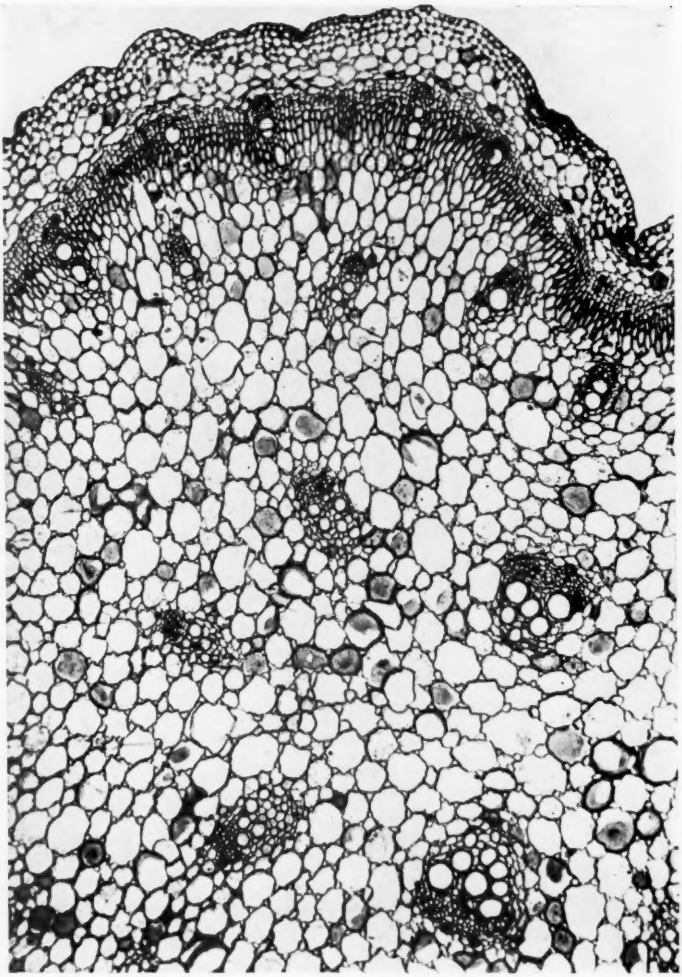

FIG. 23.—Transection of first internode of untreated plant, fourth internode of which has elongated. Secondary xylem, phloem, and conjunctive tissue derived as explained for fig. 4.

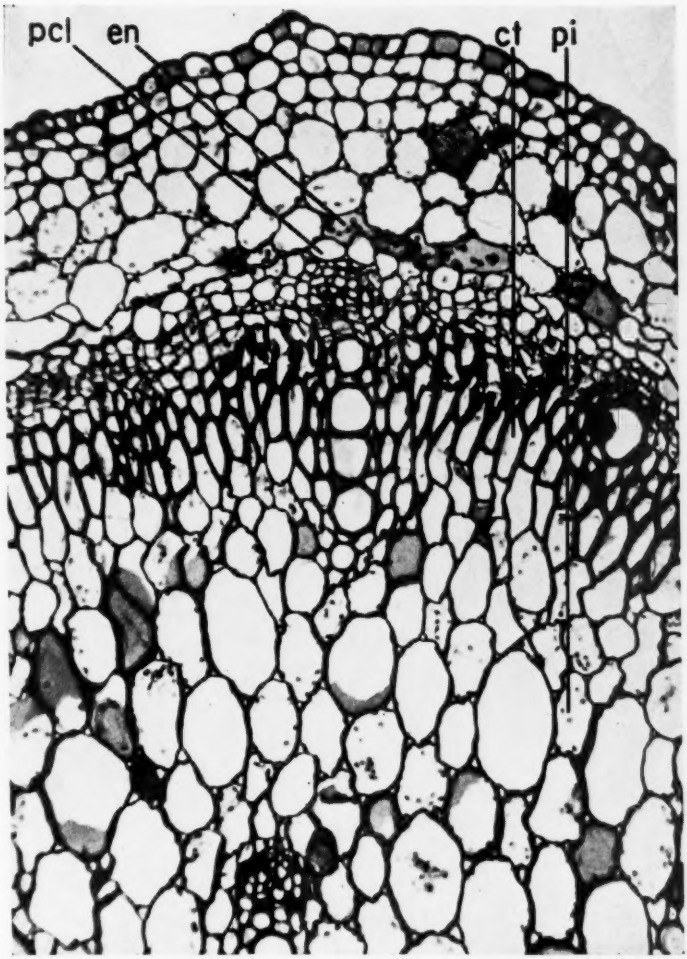

FIG. 24.—Enlarged portion as shown in fig. 23. Endodermis and pericycle not meristematic. Fascicular cambium of bundle at center continuous with interfascicular cambium derived at interfascicular parenchyma. *pcl*, pericycle; *en*, endodermis; *ct*, conjunctive tissue; *pi*, pith.

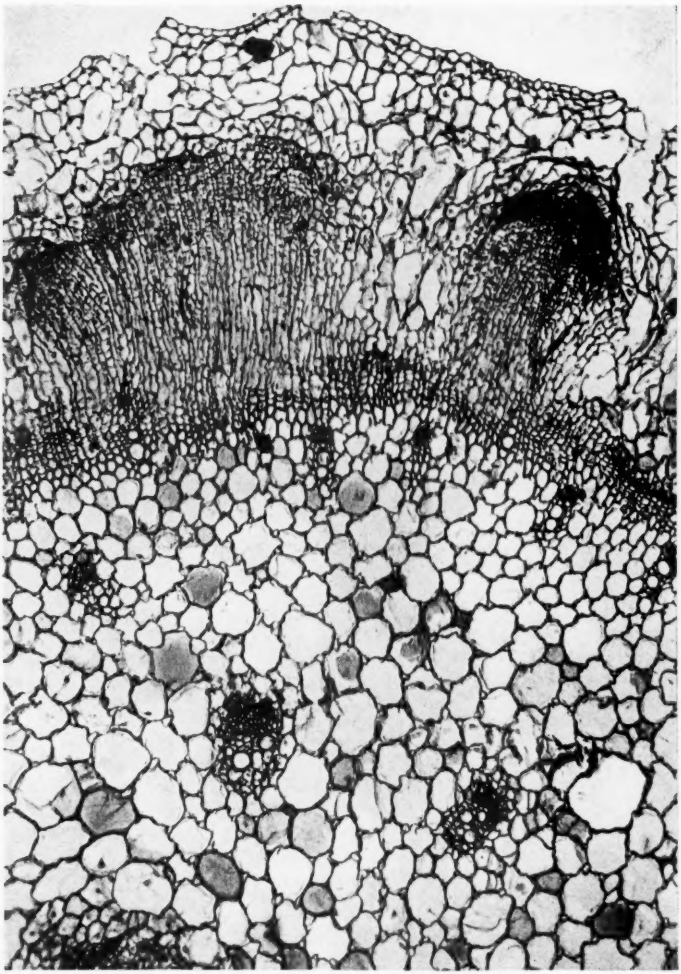

FIG. 25.—Transection of stem treated when in stage shown in figs. 23 and 24, 6 days after treatment. Tissue (except cortex and epidermis lying outside band of conjunctive tissue) shows great activity; that inside it little or no activity except for parenchymatous cells adjacent to inner vascular bundles (extreme lower left). Endodermis active.

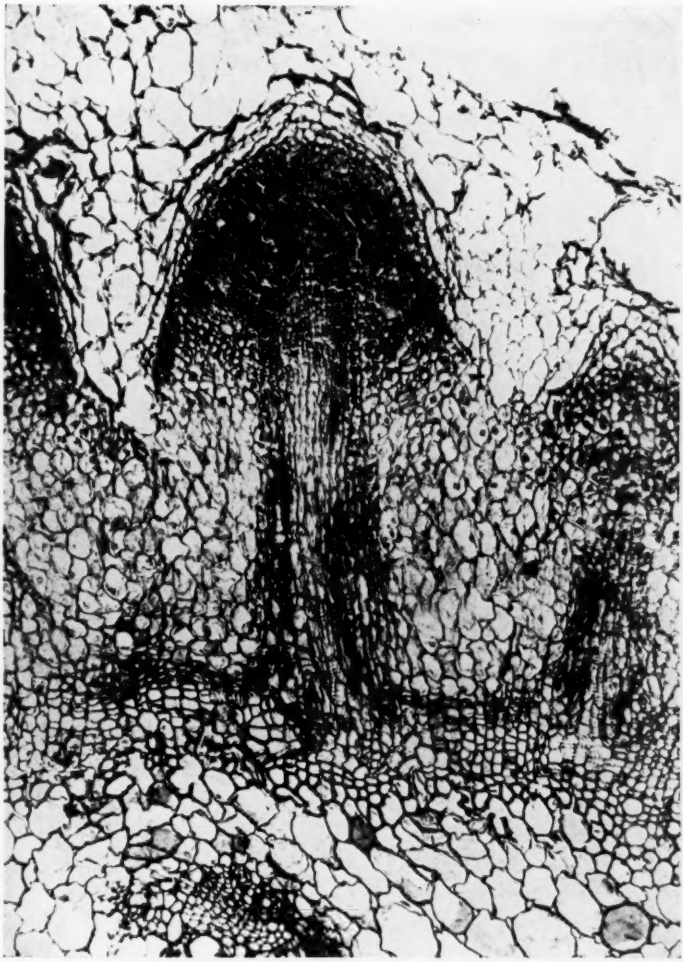

FIG. 26.—Similar to fig. 25, 11 days after treatment. Differentiation of provascular strands of root secondary to elements in band of conjunctive tissue, not to xylem nor inner vascular bundles. *Cf.* figs. 13 and 16.

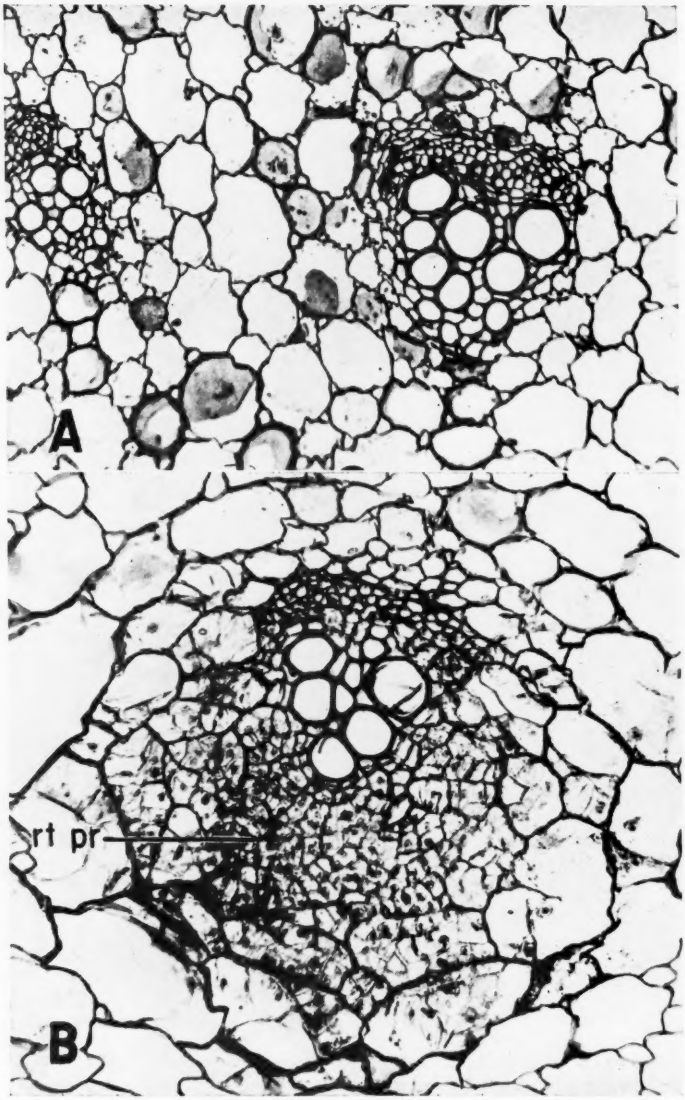

FIG. 27.—*A:* central portion of first internode of untreated plant at time fourth internode has elongated (fig. 2); pith not meristematic. Inner vascular bundle shows some secondary xylem and phloem. *B:* similar portion from plant 6 days after decapitation and treatment. Root primordium derived from proliferated parenchyma, adjacent to xylem of inner vascular bundle.

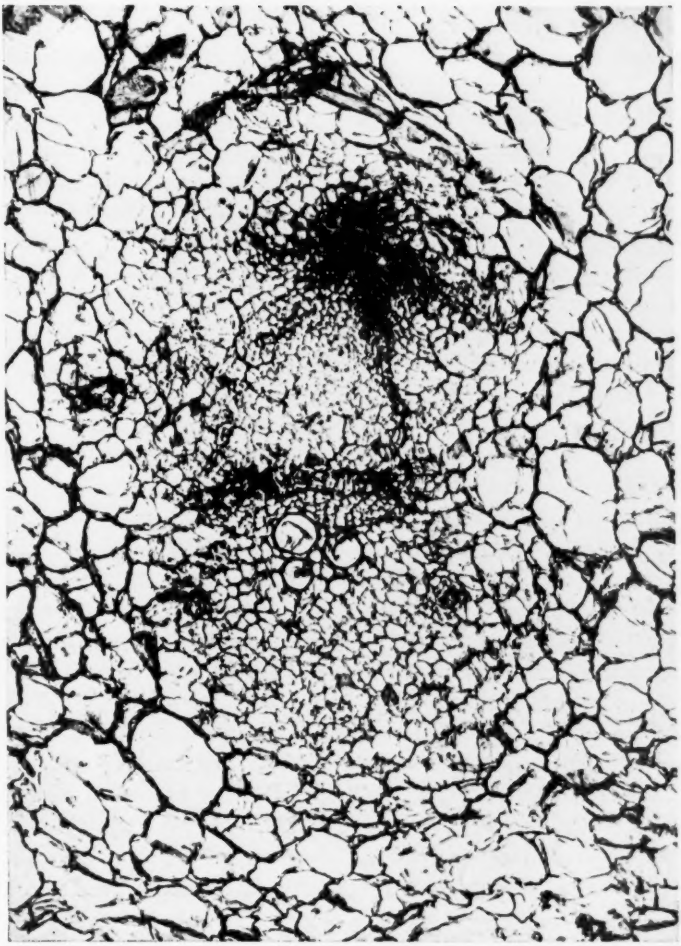

FIG. 28.—Similar to fig. 27*B*, but 11 days after treatment. Root primordium in this case derived from proliferated parenchyma over phloem of inner vascular bundle.

Discussion

In their response to indoleacetic acid in lanolin, the tissues of the stem of *Mirabilis jalapa* resemble those of the bean in the production of large tumors from the pith which may continue growth over a long period of time, but differ sharply from the bean in the far lesser activity of the endodermis, phloem, and cambium. The derived vascular bundles in the bean originated from nearly all tissues except the epidermis and cortical parenchyma, whereas in four o'clock they are derived mainly from the pith adjacent to vascular bundles already present in the stems at time of treatment.

Certain derivatives of the pith differentiate as adventitious roots, which may be directed toward the center of the axis, as was the case in *Iresine*. The epidermis and cortical parenchyma of all four plants (bean, tomato, *Iresine*, and *Mirabilis*) respond to treatment in much the same way. In its response, the endodermis of *Mirabilis* closely resembles that of tomato. The pericycle of *Mirabilis* and *Iresine* are sensitive to treatment and respond in much the same manner, in sharp contrast to the reaction of the pericycle of bean and tomato in which the pericycle is relatively unresponsive.

The responses of the vascular tissues of *Mirabilis* differ appreciably from the responses of any of the other three plants, in that the xylem, phloem, and fascicular cambium of the inner bundles at least are much less responsive. The cambium of the small outer bundles, which is continuous with the interfascicular cambium, is the principal vascular tissue which has been observed actively to divide in response to treatment.

The production of adventitious roots in tomato, *Iresine*, and *Mirabilis* is similar with respect to development and behavior. The origin of roots was in tomato from external phloem or pith parenchyma adjacent to internal phloem; in *Iresine* from pericycle or phloem or from pith parenchyma adjacent to primary xylem; and in *Mirabilis* from pericycle, interfascicular parenchyma, and pith parenchyma adjacent to primary xylem.

The effect of the age of the stem upon its response to treatment was much the same in *Mirabilis* and *Iresine*. In both plants the older stems possess a band of conjunctive tissue which seems to in-

hibit the establishment of vascular connections between the developing roots outside the band and the vascular tissue central to it. In *Iresine* the band of conjunctive tissue was observed to undergo some response to treatment; such a response was not observed in *Mirabilis*.

The fact that decapitated first internodes if untreated with the lanolin mixture are abscised in a few days, but continue to develop for long periods of time if treated, is of special interest, both in the problems of food conduction and of tissue differentiation.

The behavior of the untreated decapitated first internodes, in contrast to those treated with the lanolin mixture, is generally in keeping with those of bean, provided the latter fails to develop a callus from the phloem. SNOW (**14**) has shown a direct correlation between application of growth substances and cambial activity, and MITCHELL and MARTIN (**13**) and MITCHELL and HAMNER (**12**) have shown that although the untreated, decapitated internodes of red kidney bean are not abscised, they fail to increase markedly in volume or in dry weight, but do both when treated with indoleacetic acid-lanolin mixture. In a measure the lanolin mixture may substitute for the apical meristem and other meristematic regions which had been removed above the internode, in furnishing the stimulus which results in continued activity of the secondary meristems in the internode.

Just what the nature of this stimulus may be or how its effects are brought about still remains to be determined. The conduction of foods and nutrients as well as the multiplication, enlargement, and differentiation of cells is involved. The very low concentrations of indoleacetic acid used by MITCHELL and HAMNER, which were effective in influencing the distribution of dry weight throughout the entire bean seedling, indicate that a concentration gradient of the indoleacetic acid or some derivative from it becomes established downward from the treated surface. Such a gradient is associated with a flow of food and nutrients upward in the internode, thus enabling the tissues of the internode to grow, differentiate, and mature. The influence of the treatment is not limited to the internode just below the treated surface. In bean, adventitious roots may be produced the entire length of the stem and hypocotyl, and

other histological details are readily observable in decreasing degree away from the immediate region of application (**6, 8**).

The question arises whether the presence of additional meristems, such as root initials, buds (**2**), vascular strands, and the like, which collectively are designated as the tumor, may in themselves constitute a stimulus which causes the continued flow of nutrients to them or effects the continued differentiation of tissues below them. Such is probably not the case. Histological evidence shows that there is a decreasing gradient of activity of the various tissues away from the surface of application. As a rule tumors develop following an application of lanolin mixtures having a relatively high content of growth substance (**3, 6, 7, 8**).

There is frequently a sharp basal limitation of tumors developed following decapitation and apical application of lanolin mixture, the tissues more distant from the treated surface responding in a manner similar to those at or near the treated surfaces when dilute mixtures are applied.

The results of MITCHELL and HAMNER on bean as well as those on *Mirabilis* demonstrate clearly that tumor formation does not necessarily follow applications of low concentrations of indoleacetic acid. With certain concentrations no tumor is formed, and the development of the tissues of the decapitated treated internodes so closely parallels that of untreated undecapitated plants that histological differences between them can scarcely be detected. Thus the presence of an induced tumor, apical or otherwise, is not necessarily a primary agent in determining the course of development of tissues at distances from it; rather this is determined by the concentration of the growth substance in contact with the cell and the particular environmental conditions such as temperature, light, and moisture prevailing during the developmental period.

Summary

1. Seedlings of *Mirabilis jalapa* were decapitated at two different ages by severing the stem in the upper portion of the first internode, and the cut surface treated with a mixture of indoleacetic acid in lanolin. Similarly decapitated plants were treated with pure lanolin or untreated to serve as controls.

2. Gross observations and histological studies were made at regular intervals for several weeks on both the treated and control plants.

3. The first internode of decapitated plants fails to continue to enlarge if the cut surface is untreated or treated with lanolin only, and after about two weeks it dies. If the cut surface is treated with a 2 per cent lanolin-indoleacetic acid mixture, a tumor is formed near the top of the treated internode and the lower portion of the internode continues to enlarge.

4. There is wide variation in the histological responses of the various tissues. The pericycle, interfascicular parenchyma just inside it, and the interfascicular cambium (in the older stems) were the most responsive tissues. The vascular tissues were generally unresponsive. Epidermis and cortical parenchyma showed little response and the endodermis considerable. Cells of the pith were slow in initiation of the response but after activity had begun they proliferated rapidly and over a long period of time. Derivatives of the pith differentiated as internal roots or as strands of vascular tissue interspersed with parenchymatous cells. Activity in the pith was greatest adjacent to the vascular bundles.

5. Those roots which penetrated the cortex and epidermis were differentiated from derivatives of the pericycle or from tissue derived from both pericycle and interfascicular parenchyma or cambium.

6. The responses of the stems of *Mirabilis jalapa* are compared with those of red kidney bean, tomato, and *Iresine lindenii*.

UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS

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CURRENT LITERATURE

Genetics and the Origin of Species. By THEODOSIUS DOBZHANSKY. New York: Columbia University Press, 1937. Pp. 364. $3.60.

The enthusiasm aroused by DE VRIES'S presentation of his mutation theory was one of the principal causes of the rapid development of genetics in the early 1900's. It was widely believed that the study of mutation was about to lead to an immediate solution of the problems of the origin of species.

This early enthusiasm soon met disappointment. Much was indeed learned about heredity within species, and in certain special cases it became reasonably certain that recognized species owed their origin to a simple genetic mechanism, polyploidy. But it also became clear that the differences between closely related species are usually of a far higher order of complexity than the gene mutations or chromosome aberrations of the laboratory.

In recent years there has been an attempt to bridge the gap by treating the origin of species as a question of the transformation of populations rather than of individuals, to be approached by working out the statistical consequences of genetic mechanisms in populations subject to various conditions. This is essentially a return to DARWIN'S viewpoint but at a higher level of definiteness.

The time has been ripe for a synthesis, bringing into proper relation to one another the great stores of information which have accumulated on mutations of all sorts, on the genetic compositions of wild populations, on species differences, and on the mechanisms of hybrid sterility. It is such a synthesis that DOBZHANSKY has attempted and, in the reviewer's opinion, achieved with eminent success.

The first chapter is introductory in character. The relations of various lines of attack on the evolutionary problem are discussed. The author definitely limits the scope of his discussion to the mechanisms of speciation, excluding all consideration of the course of phylogeny, and hence of higher catagories than the species.

The second chapter deals comprehensively with gene mutation in the laboratory (types, adaptive value, multiplicity of effects, rate of occurrence under normal conditions and under special conditions, etc.). This is followed by a chapter on mutations as a basis for racial and specific differences. The author accepts the view that a race is characterized genetically by a system of gene frequencies in moving equilibrium rather than by a single typical genetic complex. The evidence for non-mendelian differentiation is discussed but the author concludes that this can hardly play more than a subordinate role. Chapter IV

gives an extensive review of types of chromosomal changes and their evolutionary significance.

After a consideration of the raw materials of evolutionary change, the statistical implications of the mendelian mechanism are introduced in chapter V. The principle of equilibrium in the absence of evolutionary pressure and the statistical effects of recurrent mutation and of inbreeding are considered and related to studies of natural populations. This is continued in the next chapter with reference to selection.

While the emphasis is on processes of gradual transformation, it is recognized that evolution is not a stereotyped process. Chapter VII deals with the evidence for abrupt speciation, as occurring rather frequently in higher plants and occasionally at least in animals, by auto- and allopolyploidy.

Chapter VIII gives a classification and discussion of the isolating mechanisms that make possible the multiplication of species. This is continued in chapter IX with respect to the problem of the nature and origin of the hybrid sterility that in most cases makes speciation an irreversible process. The contrast between sterility due to failure of pairing between differently arranged chromosomes and that due to gene effects is especially stressed. The final chapter takes an affirmative view on species as natural units, in spite of difficulties of definition.

An extraordinary amount of factual material is included but is so thoroughly digested and organized that the book is highly readable throughout. One of the most valuable features for American readers is the attention paid to European and especially Russian authors whose work has often been overlooked here. It is a book which will be a necessity for all interested in the recent development of the theory of evolution.—SEWALL WRIGHT.

A Bibliography of Eastern Asiatic Botany. By ELMER D. MERRILL and EGBERT H. WALKER. Jamaica Plain, Massachusetts: Arnold Arboretum of Harvard University, 1938. Pp. xlii+719. $12.50.

This is an imposing work involving many years in its preparation, and covering "the area from western Tibet to Japan and Formosa, including China proper, Mongolia, Manchuria, Korea, and eastern and southern Siberia, and the period from the beginning of printing, not only in Europe but also in the Orient, to the end of 1936." As might be expected from a field so vast, the author-entries are numerous, totalling more than 21,000. These were obtained by the examination of over 1200 complete or partial sets of periodicals. They represent not only the various European languages but also many articles in Chinese and in Japanese. Each entry gives, following the author's name, the exact title and bibliographic citation, also a brief but usually very helpful annotation as to the nature of the cited material. The bibliography proper, covering 550 pages, is followed by "an appendix containing references to the older Chinese and Japanese botanical works, reference lists of Chinese and Japanese

botanical periodicals, and oriental authors. In the appendix Chinese and Japanese characters are used. In the bibliography proper, as well as in the appendices, the Slavic, Chinese, and Japanese titles are translated, or both transliterated and translated," a feature that greatly enhances the value of the work for most students.

The introduction states that the main objective "has been to record those papers to which botanists who are concerned with the study of plants of this area must or should refer. Papers devoted entirely to physiology, morphology, cytology, and genetics, and those without distinct taxonomic, geographic, or economic significance are omitted." The range of treatment is comprehensive, including not only the phanerogams but also the cellular and vascular cryptogams. The point of view in selection of references is said to have been chiefly taxonomic, but users will find cited a wealth of papers dealing with exploration, history of botany, bibliography, phylogeny, pathology, agriculture, horticulture, materia medica, pharmacy, biography, and divers kindred subjects. The periodical list of more than 1200 entries contains a complete reference list of serial abbreviations, with the respective fully expanded title for each, and pertinent cross references and comment. Especially noteworthy is the series of comprehensive and detailed general, regional, and systematic indices provided near the end of the volume. These cover more than 125 pages, and make the vast array of assembled data much more useful because much more usable.

In the field of bibliographic botany, this work will long stand as a monumental contribution, as well as an eloquent tribute to the untiring zeal and astute scholarship of its two authors.—E. E. SHERFF.

Plant Ecology. By JOHN E. WEAVER and FREDERIC E. CLEMENTS. 2d ed. New York: McGraw-Hill, 1938. Pp. xxii+601. Figs. 271. $5.00.

The purpose of this revised edition is "to furnish a comprehensive textbook in accord with present-day ecological progress and a guide to workers in the numerous related fields where an intimate knowledge of plants and plant environments, whether natural or modified by man, is fundamental to progress."

The book has been enlarged by about eighty pages. About sixty new illustrations are included, and fifty of the old ones have been dropped. More than four hundred titles have been added to the bibliography.

This volume differs from the original in a number of ways. The number of chapters remains the same, but their position has been altered somewhat. The chapter on Plant Succession has been placed ahead of the discussion of the Units of Vegetation, thus forming a background for CLEMENTS' system of nomenclature of climax and seral units.

Important revisions and additions have been made in and to the chapter on Methods of Studying Vegetation, following developments in America of the last decade. The methods of the plant sociologists are given less than a page, and are dismissed as of little or insufficient value. In the chapter on Succession,

CLEMENTS' current ideas of subclimax, preclimax, postclimax, and disclimax are elucidated at length, in addition to the old material. The climax units are discussed first in the chapter on Units of Vegetation followed by the seral ones. Of CLEMENTS' recently proposed terms, only faciation and facies have been added to this chapter.

Parts of the chapter on soils have been altered considerably, and a much more comprehensive picture of soil texture and structure, of the processes of soil development, and of the major soil groups is given, interpreted in accord with modern soil science. The chapter on Reaction and Stabilization has been slightly lengthened and drastically changed, the emphasis being placed upon the reactions of vegetation of importance in conservation. This leads up to the chapter on Coaction and Conservation, which includes the more or less unrelated topics of pollination, and conservation of soil, water, and wild life. The discussions of light and adaptation to water have been modernized to a great extent, especially the treatment of xerophytes. Some authorities, however, may still find reasons for criticism.

The most interesting changes in the chapters on Plants and Plant Communities as Indicators, and Climax Formations of North America, have to do with CLEMENTS' efforts to fit his more recent terms and concepts to the climax units previously recognized. Of these, the inclusion of most of Illinois, Missouri, and Iowa in the "true prairie" climax grassland will attract most attention.

In spite of certain deficiencies and opinions which conflict with the ideas of other authorities, this book will continue to be the most valuable one-volume general text and reference work in plant ecology in use in this country.—C. E. OLMSTED.

An Ecological Glossary. By J. RICHARD CARPENTER. Norman, Oklahoma: University of Oklahoma Press, 1938. Pp. x+306. Appendix of maps and tables. $4.00.

In the words of the compiler of this glossary, his purpose, a commendable one, has been "to bring together and make available the more technical and restricted usages of terms which have been and are in the ecological literature. While no pretense is made that the list is by any means exhaustive, nearly all of the technical terms encountered in a diligent search of the current periodicals and texts are included." Examined according to this purpose, one finds that about 3000 terms are defined. A large number of the definitions are copied directly without editing or amplifying from CLEMENTS' publications of 1902, 1904, and 1905, and from JACKSON'S Glossary of Botanic Terms. Many obsolete terms used only in the publications in which they were proposed are thus included, while modern terms and modern usages are often lacking. In addition to the errors of inclusion and omission, there are numerous incorrect citations and inaccurate definitions, errors in spelling, both typographical and otherwise, and frequent failure of the cross reference system. In the opinion of the reviewer,

these marks of hasty preparation and lack of balance more than offset the real value of the book, and may hinder, rather than aid, the stabilization of ecological nomenclature and the advance of the science.—C. E. OLMSTED.

The Structure and Development of the Fungi. By H. C. I. GWYNNE-VAUGHAN and B. BARNES. 2d ed. Cambridge: University Press. New York: Macmillan Co., 1937. Pp. 449. Illustrated. $5.50.

The second edition of this well known textbook has been entirely reset and revised to include the advances in the knowledge of the fungi during the last decade. The new book contains sixty-five additional pages of text material and twenty-four new figures.

The organization remains essentially unchanged. The introduction deals with the general characters of the fungi. Several new topics and much other new material have been included in the various chapters; as, for example, the significance of flagellation as a guide to the interrelationships of the Phycomycetes, heterothallism in the rusts, and a number of new life histories. Some forty pages are devoted to the literature, covering approximately 1000 titles. The final chapter, which deals with mycological technique, should prove useful to students.

The book is characterized by its conservatism in classification and treatment. The authors apparently have been hesitant to accept some of the more significant ideas of GAUMANN: for example, the importance of the degree of development of the dicaryophase as a basis of the systematic classification of the Ascomycetes and the structure and course of development of the basidia in the Basidiomycetes. They also have failed to include some of the newer research reported in the literature of recent years. One cannot find, for instance, HANNA'S work on *Ustilago zeae*, in which he reports invasion of the host by haplophase mycelia formed by germination of the sprout cells. On the whole, however, the book is carefully and critically done, and it will continue to be a valuable text for students interested in the morphology of the fungi.—J. M. BEAL.

Catalogue of the Flora of the State of Texas. By V. L. CORY. Texas Agr. Exp. Station: Bull. 550, 1937. Pp. 130.

Valuable Plants Native to Texas. By H. B. PARKS. Texas Agr. Exp. Station: Bull. 551, 1937. Pp. 173.

The recent catalogue of the vascular plants of Texas, prepared by CORY, is the first attempt to unify the diversified and scattered taxonomic literature of this floristically important state. Technical and common names follow the International Rules of Botanical Nomenclature and the Standardized Plant Names, respectively. By means of key numbers which follow each plant name, the distribution of that species may be ascertained by consulting the simplified

map of the state. In all 162 families, 1063 genera, and 5099 species and varieties are listed. The nomenclature is in accordance with the newest researches, and many of the older, now invalid, names are given parenthetically.

PARKS has discussed the economically important plants of Texas in a recent state publication. Arranged by families in the conventional manner, nearly 1000 native plants are listed and described for their success or possible success in ornamental plantings, in erosion control, in shading, or for any other useful purpose. A very complete index is an aid to amateurs and professional botanists alike.—P. D. VOTH.

Determination of the Amino Acids. By RICHARD J. BLOCK. Minneapolis: Burgess Publishing Co., 1938. Pp. iii+85. Illustrated. $2.00.

The literature dealing with the quantitative and semiquantitative determination of the individual amino acids is very widely scattered. The methods now in use for such purposes have been compiled in this volume, which will serve a useful purpose wherever amino acids are to be determined. Unfortunately plant physiologists are not yet in a position to interpret the functions of the individual amino acids in growth processes, and determination of the mixtures found in protein hydrolysates is hardly useful; nevertheless there will be constantly increasing attention given to the nature of the nitrogen fractions now recognized as important in the physiological behavior of the organism, in proportion to the sharpening of our tools for such investigations.

There are eleven chapters, the first five of which consider related groups of amino acids together. These groups are as follows: Arginine, histidine, and lysine; tyrosine, tryptophane, dihydroxyphenylalanine, and thyroxine; proline and hydroxyproline; crystine, cysteine, and methionine; and glutamic acid, aspartic acid, and hydroxyglutamic acid.

The next five chapters cover the determination of amino acids which do not fall into group classifications. These are alanine, glycine, leucine, phenylalanine, and serine. The final chapter brings together various methods for the separation of amino acids in mixtures. Included are the methods of FISCHER, CHERBULIEZ, DAKIN, BRAZIER, and PRZYLECKI and KASPRZYK.

The apparatus diagrams are very useful. Each chapter is supplied with a bibliography. While the work is obviously a compilation, it will be appreciated for the time it will save in looking up methods; adequate critical information as to the limitations and the precautions to be employed will have to be sought in the original papers.—C. A. SHULL.

Die Pilze Mitteleuropas. Editorship H. Kniep, P. Claussen, and J. Barz. Werner Klinghardt, 1935.

Since the last mention in this journal (97:686. 1936) of Die Pilze Mitteleuropas, there have been issued numbers 17 and 18 of volume I (Boletaceae by

KALLENBACH), and numbers 4, 5, and 6 of volume II (Tremellineae by NEUHOFF and Lactarii by KNAUTH and NEUHOFF). These numbers are characterized by the same excellency as the previous ones.—G. K. K. LINK.

Die mikroscopischen Boden Pilze. By ANNELIESE NIETHAMMER. The Hague: W. Junk, 1937. Pp. 193. Illustrated.

The author of this treatise on soil fungi states that this "pioneer work" is intended to create a considerable number of workers devoted to the field of microscopic fungi of soils. The material is sketchily presented under the following chapter headings: Systematic survey of fungus forms; Distribution areas; Fungus cycles in nature; Pathology; Functions; Growth regulators; Fertilizer problems. The most significant contribution is the second chapter. The publication should be of interest and profit to specialists in soil problems.—G. K. K. LINK.

GENERAL INDEX

GENERAL INDEX

A

Abbe, E. C., Studies in the phylogeny of the Betulaceae. II 431

Acorus calamus, embryogeny 556

Addicott, Fred, Cultivation in vitro of excised pea roots 144

Allium, chromosomes 729

Armstrong, S. F., "British grasses and their employment in agriculture" 426

Ascorbic acid in Avena coleoptile 116

Avena, coleoptile, ascorbic acid content 116; polarized growth and cell studies 125

Avena test, and potassium salt of indole(3)acetic acid 408; photokymograph for analysis 470

Avery, G. S., Jr., Polarized growth and cell studies in Avena 125

B

Beal, J. M., Cytological studies in Phoenix 400; Histological responses of three species of Lilium to indoleacetic acid 881; book reviews 430, 670, 959

Bean plants, effect of indoleacetic acid on growth 171; stimulating effect of indoleacetic acid 569

Beard, Eleanor C., Some chromosome complements in the Cactaceae and a study of meiosis in Echinocereus papillosus 1

Beasley, J. O., Nuclear size in relation to meiosis 865

Betulaceae, phylogeny 431

Block, R. J., "Determination of the amino acids" 960

Bonner, D. M., Activity of the potassium salt of indole(3)acetic acid in the Avena test 408

Bonner, James, Cultivation in vitro of excised pea roots 144

Borthwick, H. A., Influence of photoperiods upon differentiation of meristems and blossoming of Biloxi soy beans 825

Bose, J. C., "Transactions of the Bose Research Institute" 425

Buell, M. F., Embryogeny of Acorus calamus 556

Burkholder, P. R., Polarized growth and cell studies in Avena 125

Burr, G. O., Synthesis of fats by green plants 773

C

Cactaceae, chromosome complements in 1

Cambial activity correlated with flowering and regeneration 854

Carpenter, J. R., "An ecological glossary" 958

Carroll, J. C., Daily periodicity of stomata in species of turf grasses 420

Chamberlain, C. J., book review 426

Chemical study of ripening process of Bosc pears 42

Clark, W. G., Ascorbic acid in the Avena coleoptile 116

Clements, F. E., "Plant ecology" 957

Connecticut, vegetation of sand plains 209

Cooper, G. O., Cytological investigations of Pisum sativum 584

Cooper, W. C., Hormones and root formation 599

Cory, V. L., "Catalogue of the flora of the state of Texas" 959

Creighton, Harriet B., Polarized growth and cell studies in Avena 125

Crossing-over in Allium 729

Cucurbita maxima, anatomy of primary structures and seedling 497

Cytological studies in the Myricaceae 387; in Sphaerocarpos 377; in Phoenix 400

D

Differentiation of the spiral vessels in Ricinus communis 69

Dobzhansky, T., "Genetics and the origin of species" 955

E

Echinocereus papillosus, meiosis in 1

Eckstein, O., "Potash deficiency symptoms" 428

Embryogeny of Acorus calamus 556
Emsweller, S. L., Crossing-over, fragmentation, and formation of new chromosomes in an Allium species hybrid 729
Excised, pea roots, cultivation in vitro 144; tomato roots 671

F

Fatigue in sensitive stigmas 877
Fats, synthesis by green plants 773
Fisher, A. R., "The design of experiments" 425
Fox, Lauretta E., Effects of temperature change on elongation rate of primary root 196
Fuller, H. J., Fatigue, summation, and daily variation of irritability in sensitive stigmas 877

G

Galinsoga ciliata, life history 543
Gardner, F. E., Histological comparison of fruits developing parthenocarpically and following pollination 355; Parthenocarpic fruits induced by spraying with growth promoting compounds 184
Gillette, N. J., Morphology of some American species of Psaronius 80
Gortner, R. A., "Selected topics in colloid chemistry" 669
Gourley, J. H., Meristems and fruit bud formation in relation to general horticultural practice 845
Grasses, daily periodicity of turf 420
Greenman, J. M., book review 667
Grove, W. B., "British stem and leaf fungi (Coelomycetes)" 670
Growth of excised roots of the tomato 671
Growth promoting substances, effect on plants 22, 125, 144, 171, 184, 301, 355, 408, 470, 569, 599, 671, 814, 840, 881, 912
Gustafson, F. G., Induced parthenocarpy 840
Gwynne-Vaughan, H. C. I., "The structure and development of the fungi" 959

H

Hamner, C. L., Stimulating effect of beta(3)indoleacetic acid on solid matter by bean plant 569
Hamner, K. C., Correlative effects of environmental factors on photoperiodism 615; Histological responses of Mirabilis jalapa to indoleacetic acid 912
Hanley, J. H., Fatigue, summation, and daily variation of irritability in sensitive stigmas 877
Harrison, B. F., Histological responses of Iresine lindenii to indoleacetic acid 301
Haskins, C. P., Note of an unusually transmitted lethal effect in x-rayed sorghum 872
Hawley, R. C., "Practice of silviculture" 429
Heald, F. D., "Introduction to plant pathology" 668
Herbst, C. C., Life history of Pelagophycus porra 339
Hill, A. F., "Economic botany" 425
Histological, comparison of fruits developing parthenocarpically and following pollination 355; responses of Iresine lindenii to indoleacetic acid 301; responses of Mirabilis jalapa to indoleacetic acid 912; responses of three species of Lilium to indoleacetic acid 881
Holly, affected by growth promoting substances 184
Hormones and root formation 599; *see* Growth promoting substances
Horticultural practice 845

I

Illumination, effect on tomato plants 412
Indoleacetic acid, effect on plants 171, 184, 301, 355, 408, 569, 599, 671, 881, 912
Iresine lindenii, histological responses to indoleacetic acid 301

J

Johnstone, G. R., Life history of Pelagophycus porra 339
Jones, H. A., Crossing-over, fragmentation, and formation of new chromosomes in an Allium species hybrid 729

K

Kraus, E. J., Histological comparison of fruits developing parthenocarpically and following pollination 355; book reviews 207, 425, 428, 429

L

Lilium, histological responses to indoleacetic acid 881
Link, G. K. K., book reviews 668, 960, 961
Longevity in Populus 529
Loomis, W. E., Relation of condensation reactions to meristematic development 814

M

Mackay, Elizabeth L., Variant chromosome numbers in Sphaerocarpos 377
Maneval, W. E., "A list of Missouri fungi" 430
Marth, P. C., Parthenocarpic fruits induced by spraying with growth promoting compounds 184
Martin, W. E., Chemical study of the ripening process of Bosc pears 42; Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants 171
Mechanism and quantitative application of the pea test 22
Meiotic studies in triploid Tulipa 103
Meristems, affected by photoperiod in soy bean 825; and fruit bud formation in relation to general horticultural practice 845; relation of condensation reactions to development 814; structural problems 803
Merrill, E. D., "Bibliography of eastern Asiatic botany" 956
Miller, E. S., Synthesis of fats by green plants 773
Mirabilis jalapa, histological responses to indoleacetic acid 912
Mitchell, J. W., Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants 171; Responses by tomato plants to artificial illumination 412; Stimulating effect of beta(3)-indoleacetic acid on synthesis of solid matter by bean plant 569
Moss, E. H., Longevity of seed and establishment of seedlings in Populus 529
Myricaceae, cytological studies 387

N

Niethammer, A., "Die mikroscopischen Boden Pilze" 961
Norman, A. G., "Biochemistry of cellulose, etc." 427
Nuclear size in relation to meiosis 865

O

Olmsted, C. E., Vegetation of certain sand plains of Connecticut 209; book reviews 957, 958
Overbeek, J. van, Mechanism and quantitative application of the pea test 22

P

Parker, M. W., Influence of photoperiod upon differentiation of meristems and blossoming of Biloxi soy beans 825
Parks, H. B., "Valuable plants native to Texas" 959
Parthenocarpic fruits induced by spraying with growth promoting compounds 184
Parthenocarpy, affected by growth promoting substances 355; induced 840
Patterns of growth of plants developed from immature embryos 630
Pea roots, cultivation in vitro 144
Pea test, mechanism and quantitative application 22
Pelagophycus porra, life history 339
Phoenix, cytological studies 400
Photokymograph for analysis of Avena test 470
Photoperiod, effect upon differentiation of meristems and blossoming of Biloxi soy beans 825
Photoperiodism, correlative effects of environmental factors 615
Phylogeny of the Betulaceae 431
Pisum sativum, cytological investigations 584
Polarized growth and cell studies in Avena 125
Popham, R. A., Life history of Galinsoga ciliata 543
Populus, seedling longevity 529
Potassium salt of indole(3)acetic acid and the Avena test 408
Prunus species, growth patterns of plants from immature embryos 630
Psaronius, morphology of 80

R

Rice plant, developmental anatomy of seedling 786
Ricinus communis, spiral vessels 69
Ripening process of Bosc pears 42
Robbins, W. J., Growth of excised roots of the tomato 671

Robinson, D. H., "Leguminous forage plants" 429
Russell, E. J., "Soil conditions and plant growth" 208

S

Salisbury, E. J., "The living garden" 429
Sande-Bakhuyzen, H. van de, "Wheat grown under constant conditions" 208
Sand plains of Connecticut, vegetation 209
Saunders, E. R., "Floral morphology, a new outlook" 426
Schmidt, Mary B., Growth of excised roots of the tomato 671
Schneider, C. L., A photokymograph for the analysis of the Avena test 470
Scott, Flora M., Differentiation of the spiral vessels in Ricinus communis 69
Sensitive stigmas 877
Sherff, E. E., "The genus Bidens" 667; book reviews 207, 424, 956
Shull, C. A., book reviews 209, 425, 427, 428, 669, 960
Sinnott, E. W., Structural problems at the meristem 803
Smith, G. M., book review 666
Smith, K. M., "A textbook of plant virus diseases" 668
Sorghum, lethal effect of x-raying 872
Soy bean, influence of photoperiod upon meristem 825
Sphaerocarpos, variant chromosome numbers 377
Stanford, E. E., "General and economic botany" 428
St. John, H., "Flora of southeastern Washington and adjacent Idaho" 424
Stokes, J., Cytological studies in the Myricaceae 387
Structural problems at the meristem 803
Swallen, J. R., book review 426
Synthesis of fats by green plants 773

T

Taylor, W. R., "Marine algae of the northeastern coast of North America" 666
Temperature, effect of changes in, on root elongation 196
Thimann, K. V., "Phytohormones" 208
Tomato, affected by artificial illumination 412; growth of excised roots 671
Tukey, H. B., Growth patterns of plants developed from immature embryos in artificial culture 630
Tulipa, meiotic studies 103

U

Ulmus fulva, macrosporogenesis and embryo development 592

V

Variant chromosome numbers in Sphaerocarpos 377
Vegetation of certain sand plains of Connecticut 209
Voth, P. D., book review 959

W

Walker, E. H., "Bibliography of eastern Asiatic botany" 956
Walker, Ruth L., Macrosporogenesis and embryo development in Ulmus fulva 592
Weaver, J. E., "Plant ecology" 957
Welton, F. A., Daily periodicity of stomata in species of turf grasses 420
Went, F. W., A photokymograph for the analysis of the Avena test 470; Mechanism and quantitative application of the pea test 22; "Phytohormones" 208
Whelden, R. M., Note of an unusually transmitted lethal effect in x-rayed sorghum 872
Whiting, A. Geraldine, Development and anatomy of primary structures in seedling of Cucurbita maxima 497
Wilton, O. Christine, Correlation of cambial activity with flowering and regeneration 854
Woods, M. W., Meiotic studies in triploid Tulipa with special reference to bridging and fragmentation 103
Wright, Sewall, book review 955

X

X-rayed sorghum, lethal effect 872

Y

Yung, C-T., Developmental anatomy of the seedling of the rice plant 786